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Strategies to Bring Conjugated Polymers into Aqueous Media

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1.1 Introduction

Conjugated polymers (CPs) are organic macromolecules with extended π-conjugation along the molecular backbone [1, 2]. Their unique optoelectronic properties that result from the highly delocalized π-electrons can be easily manipulated through modification of the conjugated backbones. As a result, CPs have been widely used in various research fields related to organic optoelectronic devices [3–5], chemo/biosensors [6, 7], and medical diagnosis and therapy [7–9]. For biology-related applications, the main obstacle is to render CPs water soluble or water dispersible. So far, mainly three strategies have been used to bring CPs into aqueous media, which include the design and synthesis of conjugated polyelectrolytes (CPEs) and neutral water-soluble conjugated polymers (WSCPs), as well as the fabrication of water-dispersible conjugated polymer nanoparticles (CPNPs) (Scheme 1.1).

CPEs are a kind of macromolecules characterized by π-conjugated backbones and ionic side chains [10]. Their solubility in aqueous media can be fine-tuned by modification of the ionic side chains. Although neutral WSCPs do not possess any charge, they have amphiphilic segments, for example, oligo(ethylene glycol) [11], that compensate for the hydrophobic nature of the conjugated backbones. These two strategies require the chemical modification of each polymer to bring them into water. A more general and straightforward method is to prepare for the CPNPs, which can in principle bring any organic soluble polymers into aqueous media [12]. To simplify our discussion, in this chapter, we only discuss CPNPs that are prepared from neutral CPs. The water solubility of CPNPs is largely determined by the polymer matrix used and the nanoparticle size, while their optical properties are associated with the neutral CP.

This chapter aims to provide readers with an overview of the strategies that can be used to bring CPs into aqueous media for potential biological applications. In this chapter, we will discuss the synthetic approaches for CPEs first, which is followed by the neutral WSCPs and CPNPs. The section on CPEs is organized

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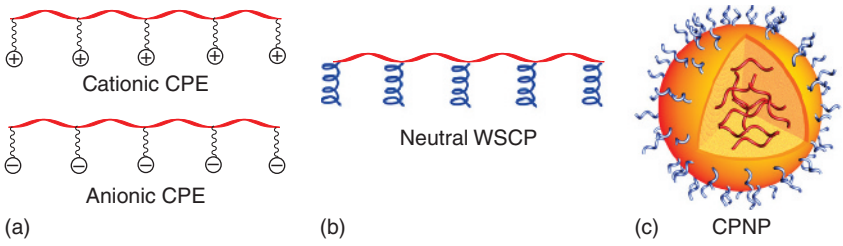
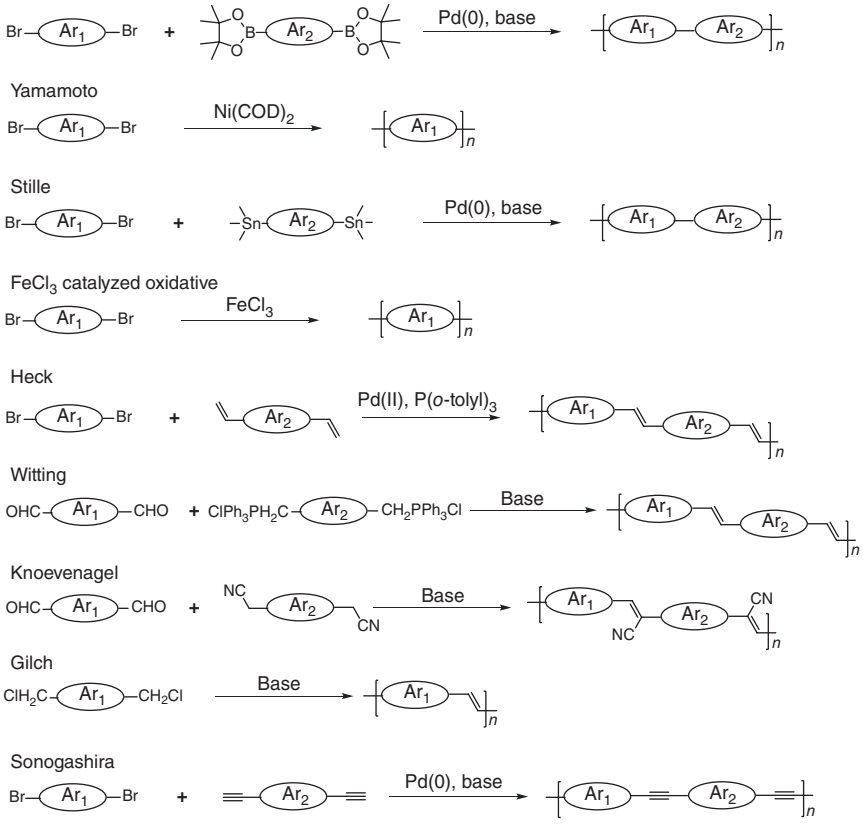


Scheme 1.1 Illustration of typical structures of CPE (a), neutral WSCP (b), and CPNP (c). The red color represents the CP backbone.

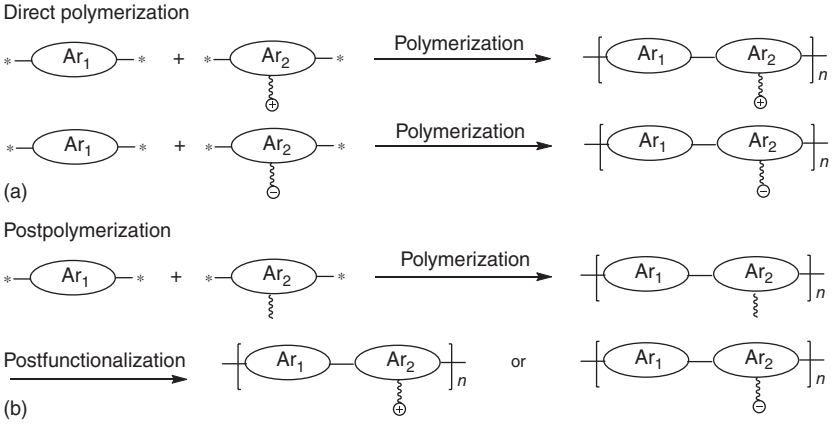
according to the charge sign. For each type of charge, we mainly select three types of CPEs (polythiophenes, poly(phenylene)s, and polyfluorenes) as examples to describe how the conjugated backbones can be synthesized and how the charged chains can be incorporated. Similar strategies will be discussed on neutral WSCPs. For the section on CPNPs, we will mainly introduce three strategies for CPNP preparation (e.g., reprecipitation, miniemulsion, and nanoprecipitation) with specific examples. Owing to the limited space, we apologize that we cannot cover every example, and only the most representative ones are selected for discussion.

1.2 Synthesis of CPEs

In the past decades, a large number of CPEs have been successfully developed. According to their chemical structures, the synthesis of CPEs involves two aspects: construction of the conjugated backbones and incorporation of charged side chains. Many well-established polymerization methods have been employed to build the conjugated backbones (Scheme 1.2), which were typically catalyzed with organometallic complexes or bases, including Suzuki, Yamamoto, Stille coupling reaction and $FeCl_3$-catalyzed oxidative reaction for single bond formation; Heck, Witting, Knoevenagel, and Gilch coupling reactions for double bond formation; and Sonogashira coupling reaction for triple bond formation. In addition, $CpCo(CO)_2$-catalyzed homopolycyclotrimerization has also been used to synthesize hyperbranched CPEs [13]. Rational design of the conjugated backbones allows facile manipulation of their optical properties, such as absorption, emission, and quantum yield. The charges can be incorporated via direct polymerization of charged monomers or postfunctionalization of neutral CPs into CPEs (Scheme 1.3). According to the charge sign of the ionic side chains, CPEs can be categorized into three groups: anionic CPEs, cationic CPEs, and zwitterionic CPEs. The anionic groups generally include sulfonate [14], carboxylate [15], and phosphonate [16], while the cationic groups include quaternary ammonium [17], pyridinum [18], and phosphonium [19]. In the following section, we will use specific examples to show the synthetic approaches of CPEs with different charges. We start each section with polythiophenes, as they are commonly synthesized via electropolymerization and $FeCl_3$-catalyzed oxidative polymerization methods. The other CPEs are generally introduced following the sequence of single, double, and triple-bonded CPEs.



Scheme 1.2 Polymerization methods most widely employed to construct conjugated backbones. Ar_1 and Ar_2 represent aromatic units.

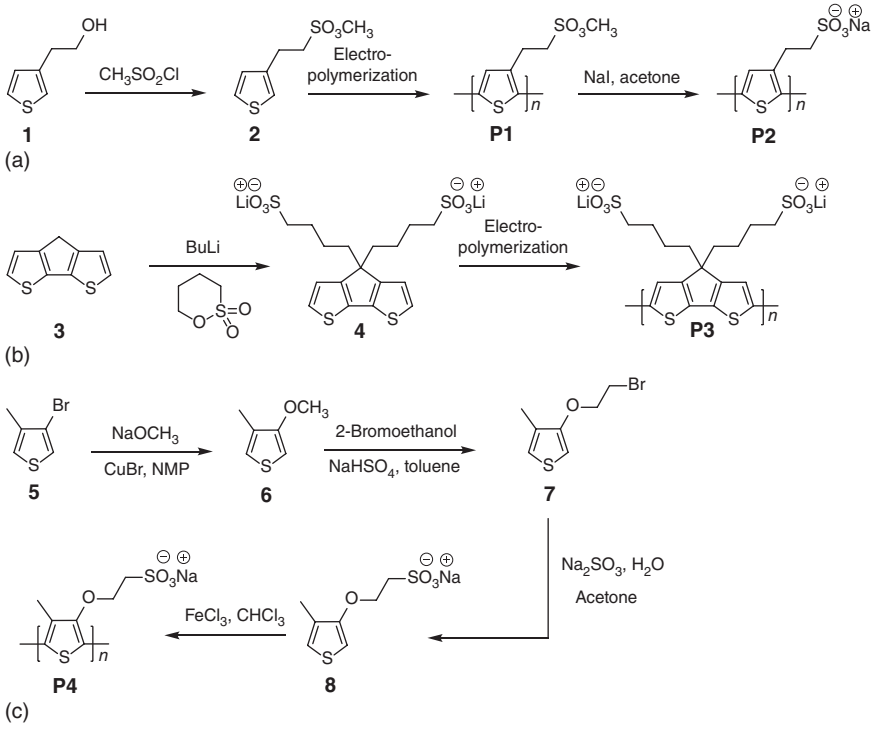


Scheme 1.3 Representative strategies for synthesis of CPEs through incorporation of charges via direct polymerization (a) and postpolymerization (b) method.

1.2.1 Anionic CPEs

1.2.1.1 Sulfonated CPEs

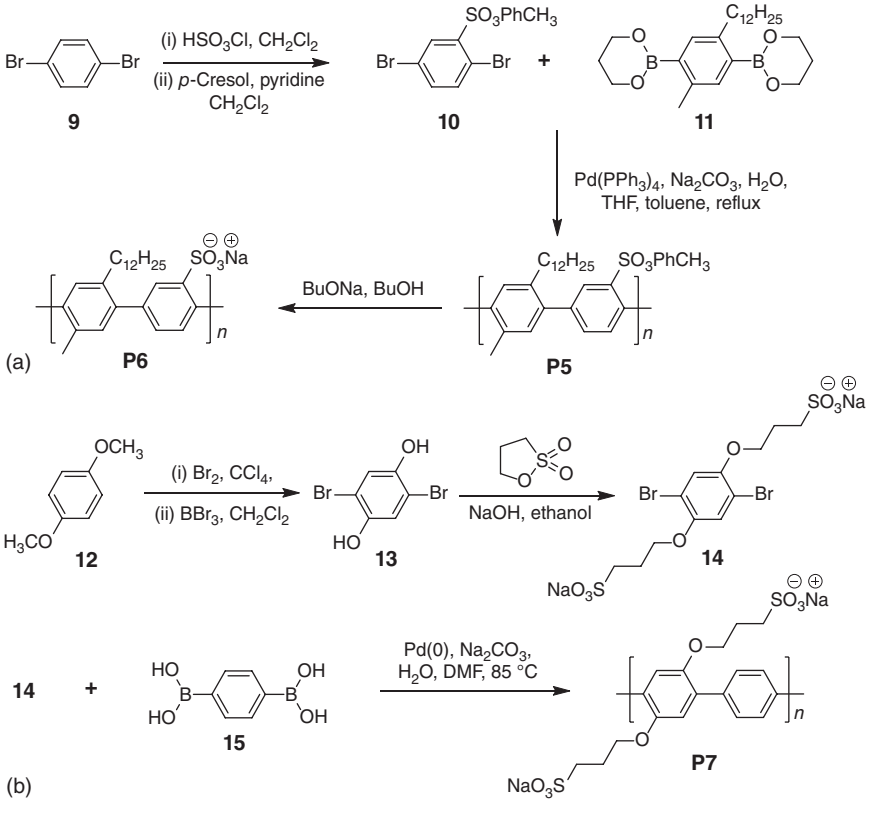
Sulfonated polythiophenes are generally synthesized through electropolymerization or $FeCl_3$-catalyzed oxidative polymerization methods. As shown in Scheme1.4a, the first sulfonated polythiophene **P2** was reported by Wudl's group in 1987 [14]. 2-(Thiophen-3-yl)ethanol **1** reacting with methanesulfonyl chloride yielded methyl 2-(thiophene-3-yl)ethanesulfonate **2**. Electropolymerization of **2** led to a neutral polythiophene **P1**, which was subsequently treated with NaI in acetone to give sulfonated **P2**. Using the same electropolymerization method, another sulfonated polythiophene **P3** was developed by Zotti's group (Scheme 1.4b) [20]. The key sulfonated monomer **4** was synthesized by alkylation of 4*H*-cyclopenta[2,1-*b*:3,4-*b*′] dithiophene **3** in the presence of 1,4-butanesultone and *n*-BuLi. The direct electropolymerization of **4** afforded **P3**. Unlike **P2** prepared via postpolymerization strategy, the sulfonated groups of **P3** are inherited from the key monomer **4**. In addition, Leclerc's group reported a sulfonated polythiophene **P4** by oxidative polymerization method (Scheme 1.4c) [21]. Briefly, the alkoxylation of 3-bromo-4-methylthiophene **5** was performed in *N*-methyl-2-pyrrolidone in the presence of sodium methoxide and copper bromide, leading to 3-methoxy-4-methylthiophene **6**, which reacted with 2-bromoethanol and sodium sulfite in toluene to yield 3-(2-bromoethoxy)-4-methylthiophene **7**. Subsequent treatment of **7** with sodium



Scheme 1.4 Synthesis of sulfonated polythiophenes **P2–P4**.

sulfite in a mixture of water/acetone led to sodium 2-(4-methyl-3-thiophenyl-1-oxy) ethanesulfonate **8**, which underwent $FeCl_3$-catalyzed oxidative polymerization to afford **P4**.

To synthesize CPEs with single bonded backbones, Suzuki polymerization was often used due to its high reaction yield and good selectivity toward various functional groups [22]. As shown in Scheme 1.5a, the first sulfonated poly(*p*-phenylene) **P6** was synthesized by Wegner's group via the Suzuki polymerization method [23]. The key monomer **10** was prepared via the chlorosulfonation of 1,4-dibromobenzene **9** with chlorosulfonic acid in dichloromethane, followed by treatment with *p*-cresol in the presence of pyridine. Then, Pd-catalyzed Suzuki polymerization between **10** and 2,2′-(2-dodecyl-5-methyl-1,4-phenylene)bis(1,3,2-dioxaborinane) **11** in the presence of sodium carbonate yielded a neutral poly(*p*-phenylene) **P5**. Subsequent solvolysis of **P5** in a mixture of sodium butanolate/1-butanol followed by the addition of water, gave the sulfonated poly(*p*-phenylene) **P6** in quantitative yield. Only one of the two possible positional isomeric structures of the repeated unit is shown in **P5** and **P6** for the sake of simplicity in illustration.



Scheme 1.5 Synthesis of sulfonated CPEs **P6** and **P7** with single-bonded backbones through Suzuki polymerization method.

A direct polymerization method for synthesizing sulfonated poly(*p*-phenylene) **P7** through Suzuki polymerization was reported by Reynolds's group via three steps (Scheme 1.5b) [24]. 2,5-Dibromohydroquinone **13** was synthesized via bromination of 1,4-dimethoxybenzene **12** using bromine in tetrachloromethane, followed by the treatment with boron tribromide in anhydrous dichloromethane. Subsequent sulfonation of **13** with 1,3-propanesultone and sodium hydroxide in absolute ethanol led to the key sulfonated monomer **14**, which directly reacted with 1,4-phenylenediboronic acid **15** through Suzuki polymerization to yield **P7**. Unlike **P5** and **P6**, there is no isomeric structure for **P7** due to its symmetric chemical structure.

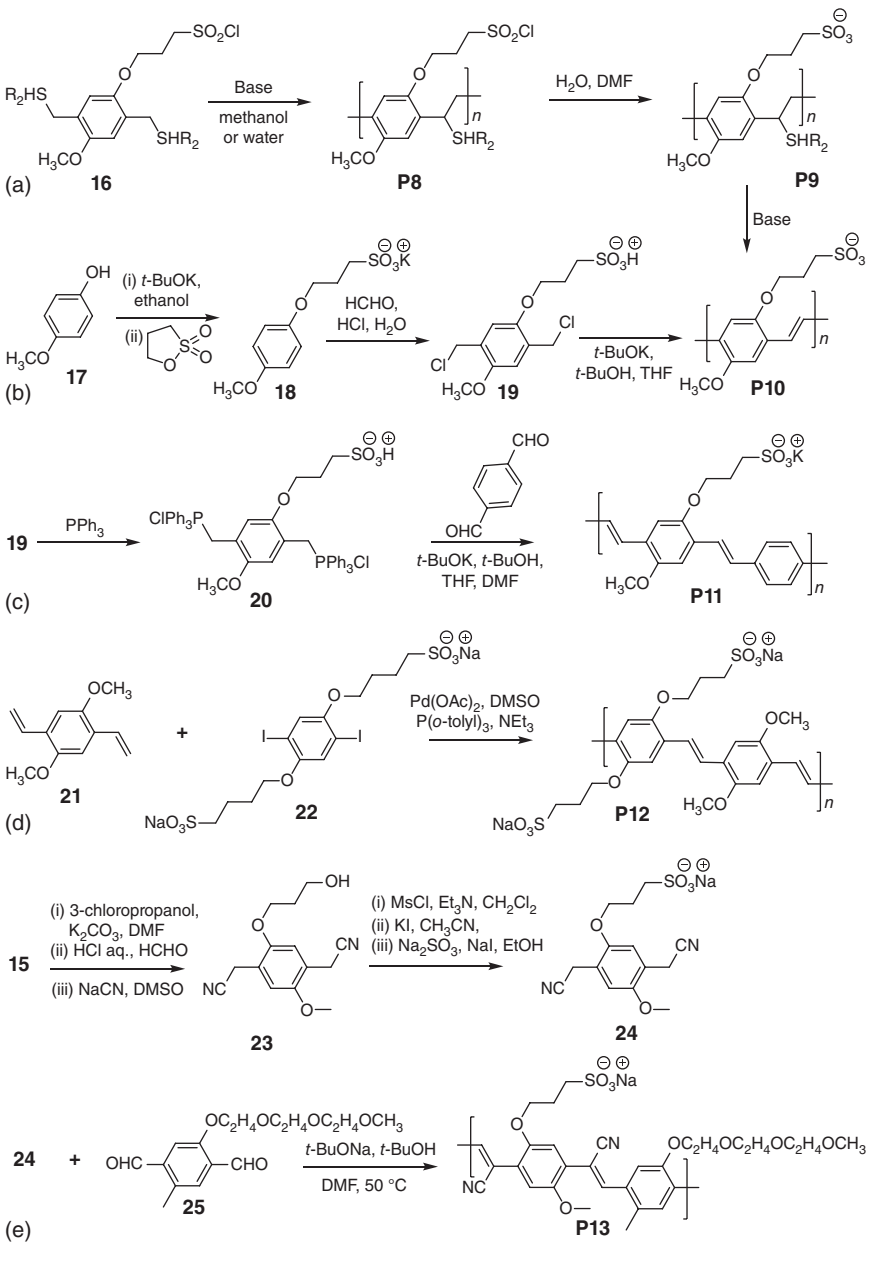
To synthesize sulfonated CPEs with double-bonded backbones, various polymerization methods have been employed. Herein, we choose the widely studied poly(*p*-phenylenevinylene)s as the examples. The first sulfonated poly(*p*-phenylenevinylene) **P10** was synthesized by Wudl's group in 1990 [25], starting from the key monomer **16** (Scheme 1.6a). **16** was self-polymerized either in methanol or in water with the help of sodium methoxide or sodium hydroxide, respectively, yielding a reactive *p*-xylylene intermediate **P8**. Further hydrolysis followed by base-assisted elimination led to the sulfonated poly(phenylene vinylene) **P10**. The purity of **16** is very crucial to ensure that **P10** can be obtained with narrow polydispersity and high molecular weight.

Another approach to synthesize **P10** was reported by Gu *et al*. [26] As shown in Scheme 1.6b, potassium 3-(4-methoxyphenoxy)propanesulfonate **18** was prepared through the esterification of 4-methoxyphenol **17** with 1,3-propanesultone in anhydrous ethanol under basic conditions. Further chloromethylation of **18** with paraformaldehyde in acidic aqueous at 40 °C led to the key monomer **19**. Gilch dehydrohalogenation polymerization of **19** was performed using *t*-BuOK as catalyst to give **P10**.

The same group also employed Witting polymerization to synthesize poly(*p*-phenylenevinylene) derivatives (Scheme 1.6c) [27]. A mixture of **19** and triphenylphosphine in anhydrous toluene was kept at reflux conditions leading to the monomer **20**, which was copolymerized with terephthalaldehyde in *tert*-butyl alcohol through Witting condensation with potassium *tert*-butoxide as catalyst to yield alternating sulfonated poly(*p*-phenylenevinylene) **P11**.

Heck polymerization is also an important approach to synthesize sulfonated poly(*p*-phenylenevinylene) derivatives. As shown in Scheme 1.6d, the sulfonated diiodo-substituted monomer **22**, which was synthesized with a similar approach as for **18**, copolymerized with 1,4-dimethoxy-2,5-divinylbenzene **21** using palladium acetate and tri(*o*-tolyl)phosphine as cocatalysts in dimethyl sulfoxide under basic conditions affording the alternating sulfonated poly(*p*-phenylenevinylene) **P12** [28].

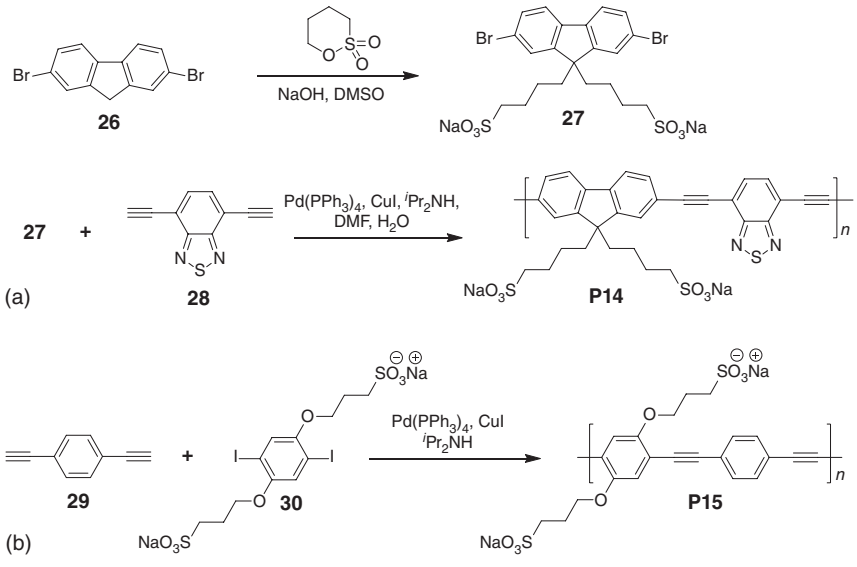
In addition to the abovementioned approaches for synthesizing poly(*p*-phenylenevinylene)s, Knoevenagel polymerization has also been used to prepare cyano-substituted poly(*p*-phenylenevinylene) derivatives [29]. As shown in Scheme 1.6e, 4-methoxyphenol **17** subsequently underwent alkylation, chloromethylation, and cyanide exchange reactions to yield **23**. Subsequently, the hydroxyl groups of **23** underwent sequential methanesulfonylation, iodination, and sulfonation, affording the key monomer **24**. Knoevenagel condensation reaction between **24** and the neutral dialdehyde monomer **25** using *t*-BuONa as catalyst in a mixture of *t*-BuOH/DMF led to the sulfonated **P13**.



Scheme 1.6 Synthesis of sulfonated CPEs **P10–P13** with double-bonded backbones.

To synthesize CPEs with triple-bonded backbones, Sonogashira coupling reaction is commonly employed. This reaction involves a palladium-catalyzed sp^2–sp coupling reaction between aryl or alkenyl halides or triflates and terminal alkynes, with or without a copper(I) cocatalyst [30]. It can be performed under mild conditions, such as room temperature, in aqueous media. Several

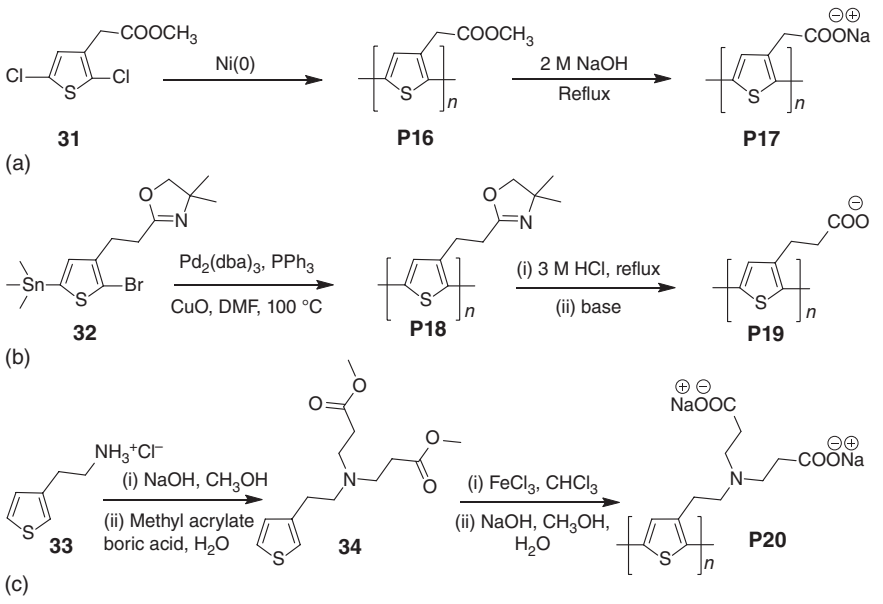
monomers could also be synthesized with sulfonate functional groups. A direct polymerization approach to synthesize sulfonated polyfluorene **P14** was reported by Liu's group (Scheme 1.7a). It only requires one step to synthesize the key monomer 2,7-dibromo-9,9-bis(4-sulfonatobutyl)fluorene **27** via direct alkylation of 2,7-dibromofluorene **26** with 1,4-butane sultone and NaOH in DMSO [31]. The copolymerization of **27** and 4,7-diethynyl-2,1,3-benzothiadiazone **28** under Sonogashira coupling reaction conditions led to the sulfonated poly(fluorene vinylene) **P14** [32]. In addition, as shown in Scheme 1.7b, the key sulfonated monomer **30** can be prepared using procedures similar to **18** and **22**. Sonogashira reaction between 1,4-diethynylbenzene **29** and **30** in basic aqueous/DMF solution using tetrakis(triphenylphosphine)palladium and copper iodide as co-catalysts led to **P15**. In 2006, by utilizing the above mentioned strategy, Schanze's group developed a series of sulfonated poly(phenylene ethynylene)s with variable band gaps based on the monomer **30** [33].



Scheme 1.7 Synthesis of sulfonated CPEs **P14** and **P15** with triple-bonded backbones through Sonogashira reaction.

1.2.1.2 Carboxylated CPEs

Carboxylated polythiophenes have been prepared through Yamamoto coupling polymerization, Stille coupling polymerization, and $FeCl_3$-catalyzed oxidative polymerization. As shown in Scheme 1.8a, the neutral poly(methyl thiophene-3-carboxylate) **P16** was synthesized via the Yamamoto polymerization of methyl 2-(2,5-dichlorothiophen-3-yl)acetate **31** [15]. Subsequently, the hydrolysis of **P16** in 2.0 M NaOH aqueous led to poly(sodium thiophene-3-carboxylate) **P17**. During the purification process, filtration was needed to remove the insoluble fraction. In addition, a CuO-mediated Stille coupling polymerization of **32** was carried out to give poly(4,5-dihydro-4,4-dimethyl-2-(2-(thiophen-3-yl)ethyl)

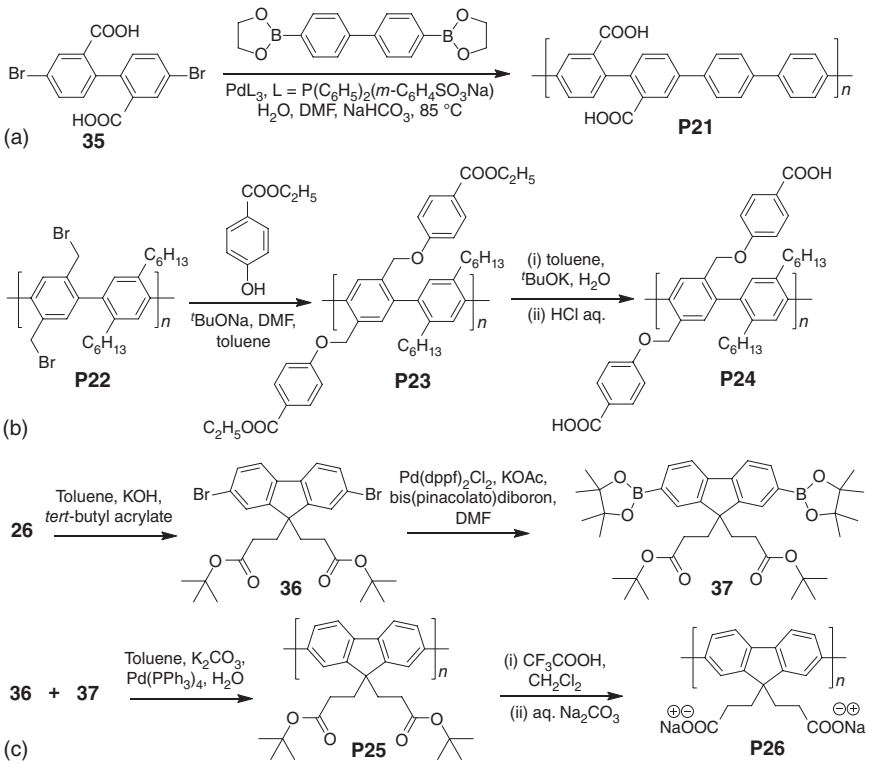


Scheme 1.8 Synthesis of carboxylated polythiophenes **P17**, **P19**, and **P20**.

oxazole) **P18** (Scheme 1.8b) [34], which was converted to **P19** after acid-assisted hydrolysis and base treatment. Compared to **P17** and **P19**, which only have one carboxylate group on each repeat unit, **P20** reported by Wang's group possesses two carboxylate groups on each repeat unit (Scheme 1.8c) [35]. The monomer **34** was synthesized by reacting 2-(3-thienyl)ethylamine hydrochloride **33** with methyl acrylate under basic conditions in the presence of boric acid. Oxidative polymerization of **34** in chloroform using $FeCl_3$ as oxidizing agent followed by hydrolysis in NaOH aqueous solution yielded **P20**.

Suzuki polymerization is generally used to synthesize carboxylated CPEs with single-bonded backbones. The first carboxylated poly(*p*-phenylene) **P21** was reported by Wallow and Novak in 1991 [36]. As shown in Scheme 1.9a, Pd(0)-catalyzed Suzuki coupling reaction between 4,4′-dibromo-[1,1′-biphenyl]-2,2′-dicarboxylic acid **35** and 4,4′-di(1,3,2-dioxaborolan-2-yl)-1,1′-biphenyl followed by treatment with dilute hydrochloric acid yield **P21** with free acid. **P21** was completely insoluble in all common organic solvents, but was soluble in dilute aqueous base as its sodium, potassium, or triethylammonium salt.

A postfunctionalization approach to synthesize carboxylated poly(*p*-phenylene)s was later reported by Rehahn's group starting from the precursor polymer **P22** (Scheme 1.9b) [37]. Owing to the high reactivity of bromide methylene group on **P22**, etherification of **P22** with ethyl *p*-hydroxyl benzoate in a mixture of toluene/DMF in the presence of *t*-BuONa produced **P23** in nearly quantitative yield. During the reaction process, the ester groups were kept intact. Almost all the ester groups of **P23** was cleaved in a homogeneous solution of toluene in the presence of 10 equiv. of *t*-BuOK and only 2 equiv. of water, followed



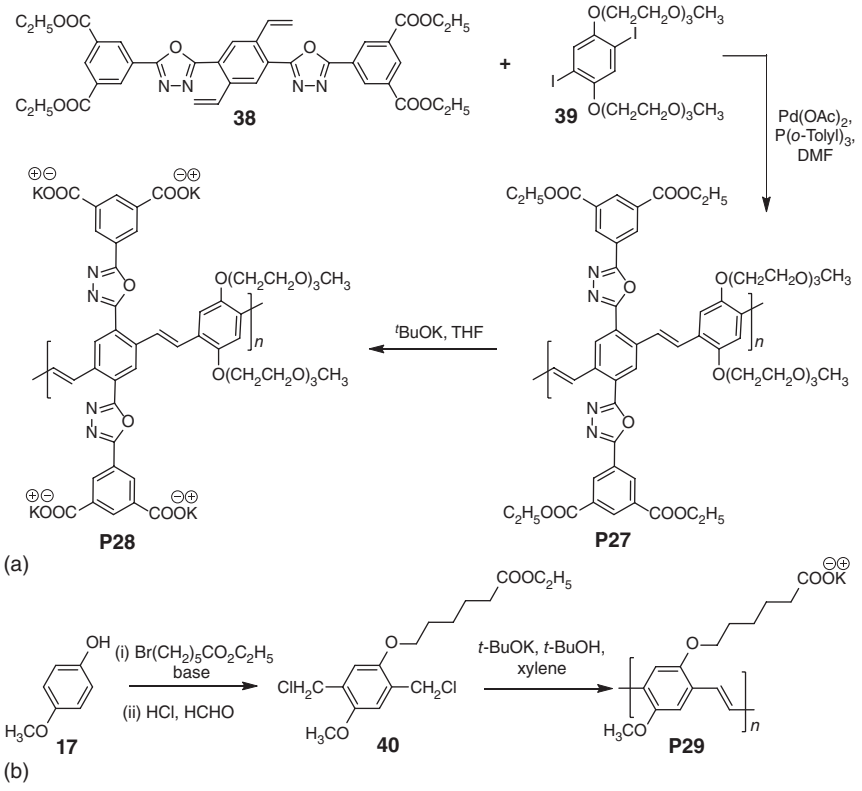
Scheme 1.9 Synthesis of carboxylated CPEs **P21**, **P24**, and **P26** with single-bonded backbones.

by acidification with hydrochloric acid to yield **P24**. However, unexpectedly, no hydrolysis of **P23** was observed in a two-phase system of water/toluene in the presence of NaOH.

Another postpolymerization approach to synthesize carboxylated polyfluorene was reported by Liu's group (Scheme 1.9c) [38]. Direct alkylation of **26** via Michael addition of the 9-position carbon with *tert*-butylacrylate afforded fluorene ester **36**, which was converted to the corresponding diboronate ester **37** under Suzuki–Miyaura reaction conditions in the presence of bis(pinacolato) diborane, $Pd(dppf)_2Cl_2$, and KOAc with anhydrous DMF as the solvent. In the next step, Suzuki polymerization between **36** and **37** led to the neutral precursor polymer **P25**. Contrary to the preparation of **P24** where the hydrolysis was performed under basic condition, **P26** was obtained by the hydrolysis of **P25** in acid condition followed by the neutralization with aqueous Na_2CO_3.

Carboxylated CPEs with double-bonded backbones have been synthesized through Heck and Gilch polymerization methods. As shown in Scheme 1.10a, Heck coupling polymerization between **38** and the diiodo-substituted monomer **39** in DMF using $Pd(OAc)_2$ and P(*o*-Tolyl)$_3$ as cocatalysts yielded the precursor polymer **P27**, which was hydrolyzed into carboxylated **P28** in THF with *t*-BuOK

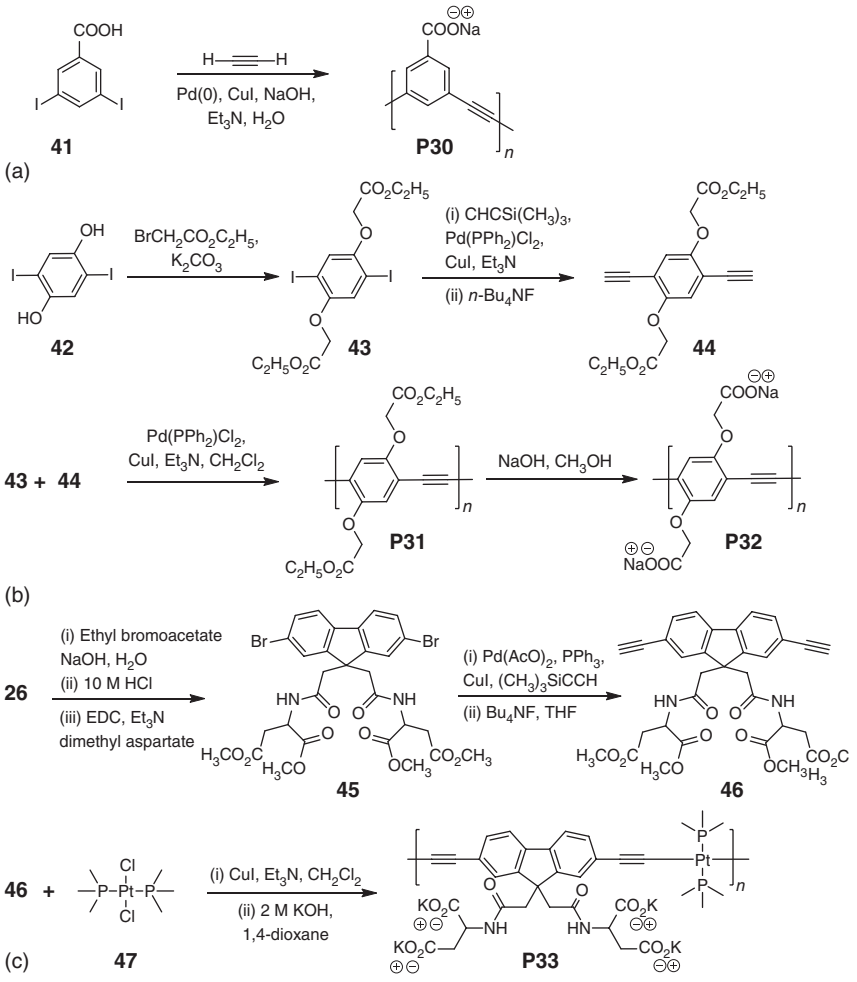
as the base [39]. **P28** is only soluble in DMSO and dilute aqueous bases such as NaOH and NH_4OH but is insoluble in $CHCl_3$. Similarly, Gilch dehydrochlorination polymerization was also used to synthesize carboxylated poly(phenylene vinylene) **P29** (Scheme 1.10b) [40]. The key monomer **40** was prepared by etherification of 4-methoxyphenol **17** with 1-bromohexanoic acid ethyl ester and MeONa, followed by chloromethylation with formaldehyde and hydrochloric acid. In the next step, Gilch dehydrochlorination polymerization of **40** in the presence of *t*-BuOK yielded the carboxylated **P29**.



Scheme 1.10 Synthesis of carboxylated CPEs **P28** and **P29** with double-bonded backbones.

Sonogashira coupling reaction is the most commonly used method to synthesize carboxylated CPEs with triple-bonded backbones. As shown in Scheme 1.11a, **P30** was directly obtained by polymerization of 3,5-diiodo benzoic acid **41** with acetylene gas in water in the presence of a water-soluble Pd(0) catalyst, CuI co-catalyst, 1 equiv. of NaOH and 3 equiv. of Et_3N [41].

Bunz's group synthesized **P32** by a postpolymerization method (Scheme 1.11b) [42]. Starting from 2,5-diiodohydroquinone **42**, reaction with 2-bromoethyl acetate in butanone in the presence of K_2CO_3 yielded the ester-protected diiodo-monomer **43**. Subsequent alkylation utilizing trimethylsilylacetylene and the catalysts of $Pd(PPh_3)_2Cl_2$/CuI with trimethylamine as the solvent



Scheme 1.11 Synthesis of carboxylated CPEs **P30**, **P32**, and **P33** with triple-bonded backbones.

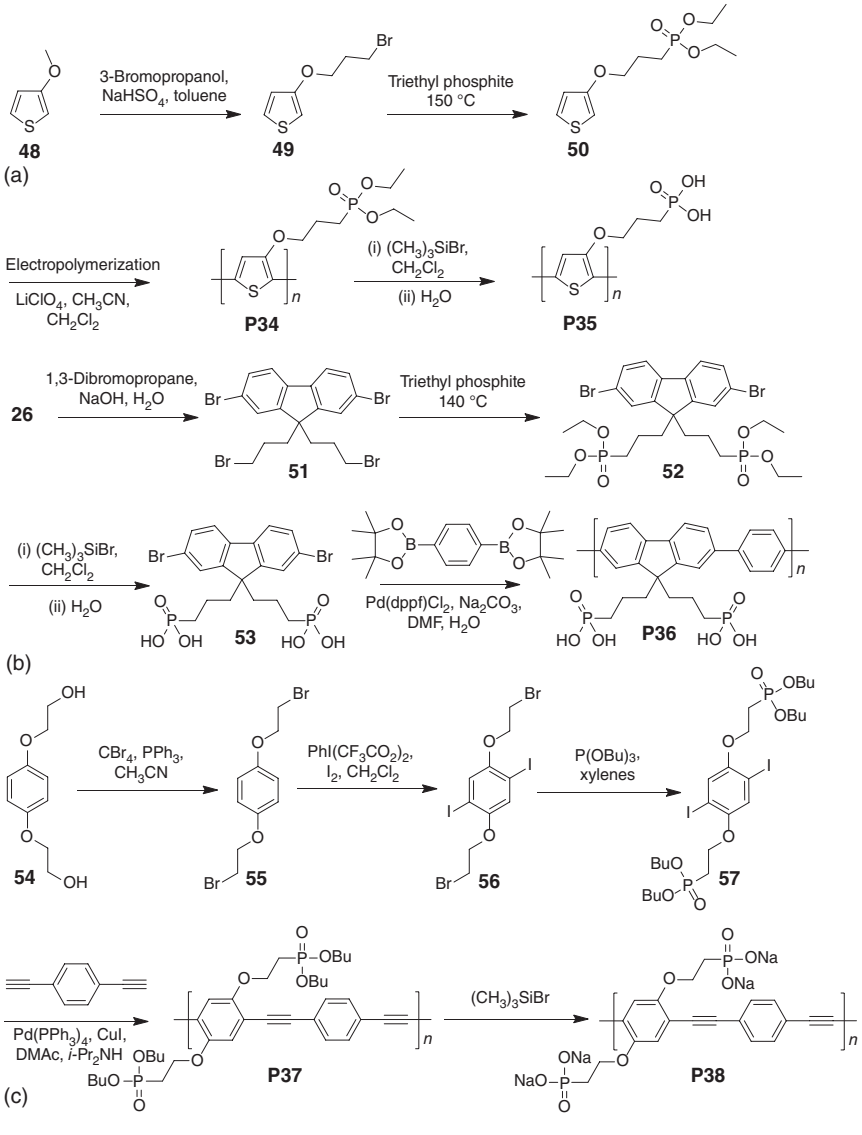
furnished **44** after desilylation with tetrabutylammonium fluoride in THF. It is important to note that the ester groups were not cleaved under such basic conditions. Copolymerization between **43** and **44** under Sonogashira reaction conditions yielded the neutral **P31**, which was converted to **P32** through hydrolysis with NaOH in methanol [42].

To increase the number of carboxylate groups on each repeat unit, Wang's group reported **P33** from the key monomer **46** (Scheme 1.11c). Starting from the alkylation of 2,7-dibromofluorene **26** with ethyl bromoacetate under basic conditions followed by treatment with HCl solution, the obtained intermediate further reacted with L-aspartic acid dimethyl ester hydrochloride via *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) hydrochloride-catalyzed coupling reaction at room temperature to yield **45** [43]. The key monomer **46** can be easily obtained by reaction between **45** and trimethylsilylacetylene using $Pd(PPh_3)_2Cl_2$/CuI as catalyst

followed by desilylation with tetrabutylammonium fluoride in THF. Subsequently, Sonogashira coupling reaction between **46** and $Pt(PMe_3)_2Cl_2$ **47** followed by hydrolysis of the ester groups using 2 M KOH solution as the base yielded the desired carboxylated **P33** with four carboxylate groups on each repeat unit [44].

1.2.1.3 Phosphonated CPEs

As compared to sulfonated and carboxylated CPEs, the studies concerning phosphonated CPEs are less reported [16, 45, 46]. As shown in Scheme 1.12a, 3-(3-bromopropoxy)thiophene **49** was synthesized from 3-methoxythiophen **48**



Scheme 1.12 Synthesis of phosphonated CPEs **P35**, **P36**, and **P38**.

and 3-bromopropanol in toluene in the presence of $NaHSO_4$. Treatment of **49** with triethyl phosphite yielded the phosphonic acid diethyl ester **50**. Direct electropolymerization of **50** led to the precursor polymer **P34**, which was hydrolyzed into phosphonated **P35** using bromotrimethylsilane [16].

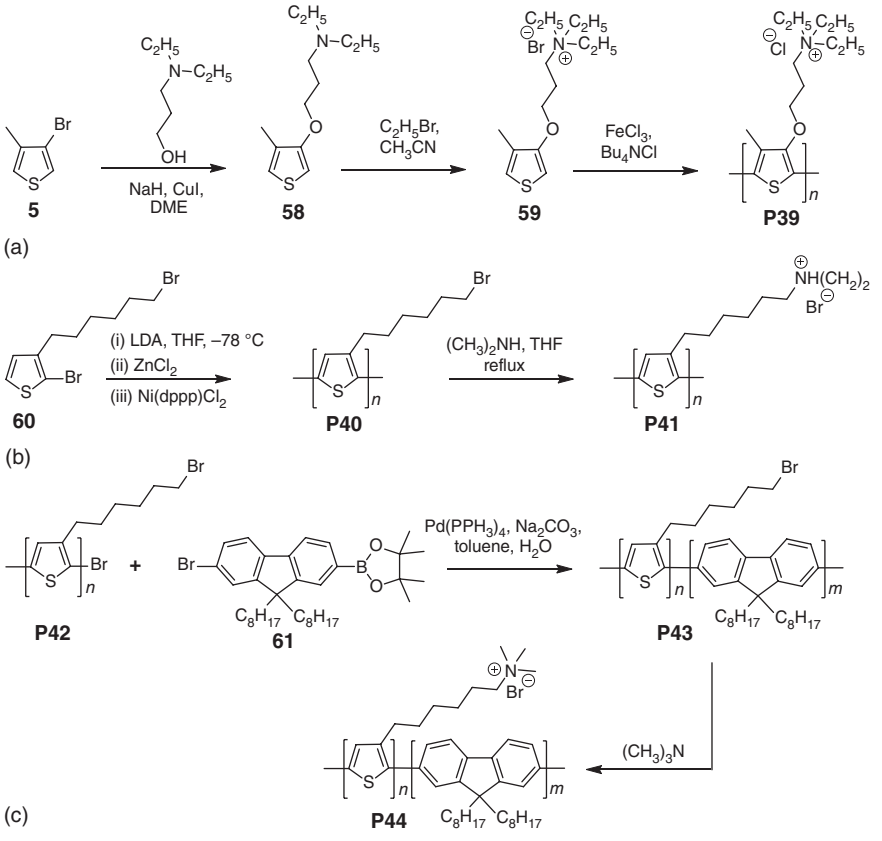
A phosphonated polyfluorene **P36** was also synthesized by Wang's group via direct Suzuki polymerization method (Scheme 1.12b) [46]. Starting from 2,7-dibromofluorene **26**, reaction with 1,3-dibromopropane in aqueous NaOH yielded 2,7-dibromo-9,9-bis(3-bromopropyl)fluorene **51**, which reacted with triethylphosphite to afford **52** in a quantitative yield. Treatment of **52** with trimethylsilyl bromide and subsequently water yielded 2,7-dibromo-9,9-bis(3-diethoxylphosphorylpropyl)fluorene **53**. Direct Suzuki polymerization between **53** and 1,4-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene using $Pd(dppf)Cl_2$ as catalyst in DMF and Na_2CO_3 aqueous solution gave **P36** with molecular weight higher than 15 kDa.

Phosphonated CPE **P38** with triple-bonded backbones was also synthesized. As shown in Scheme 1.12c, bromination of **54** in acetonitrile using carbon tetrabromide and triphenylphosphine yielded **55**, which underwent iodination to give the compound **56**. The key monomer **57** was synthesized from **56** using a similar procedure to **50** and **52**. **P38** was then prepared via Sonogashira reaction between **57** and 1,4-diethynybenzene to yield **P37**, which was followed by trimethylsilyl bromide treatment to promote the hydrolysis of the butylphosphonate ester groups (Scheme 1.12c) [45]. However, Sonogashira reaction between the hydrolysis product of **57** and 1,4-diethynybenzene could not afford high molecular weight for **P38**, presumably because the ionic phosphonate groups can coordinate with and deactivate the catalysts during the polymerization.

1.2.2 Cationic CPEs

1.2.2.1 Ammonium CPEs

Ammonium polythiophene **P39** was reported by Leclerc's group through direct oxidation of cationic thiophene monomer **59** (Scheme 1.13a) [47]. The key monomer **59** was prepared from the Williamson reaction between 3-bromo-4-methylthiophene **5** and 3-(diethylamino)propanol to yield **58**, which was followed by quaternization with bromoethane. Oxidative polymerization of **59** in chloroform using $FeCl_3$ as the oxidizing agent yielded **P39**. Alternatively, a postpolymerization method to synthesize ammonium polythiophene was also reported by McCullough's group [48]. As shown in Scheme 1.13b, a neutral polythiophene **P40** was synthesized from 2-bromo-3-hexylbromothiophene **60** via $Ni(dppp)Cl_2$ catalyzed coupling reaction. Quaternization of **P40** with methylamine in a mixture of THF/methanol yielded the cationic **P41**. It should be pointed out that the chemical structure of **P40** should be written as **P42** with a bromide on the conjugated backbone terminal, according to the reaction. Using **P42** as a macromonomer and 2-bromo-(9,9-dioctylfluorene)-7-pinacolato boronate **61** as AB-type monomer under Suzuki cross-coupling conditions, Scherf's group has successfully synthesized all-conjugated cationic diblock copolythiophenes **P44** through a "grafting from" approach (Scheme 1.13c) [49].

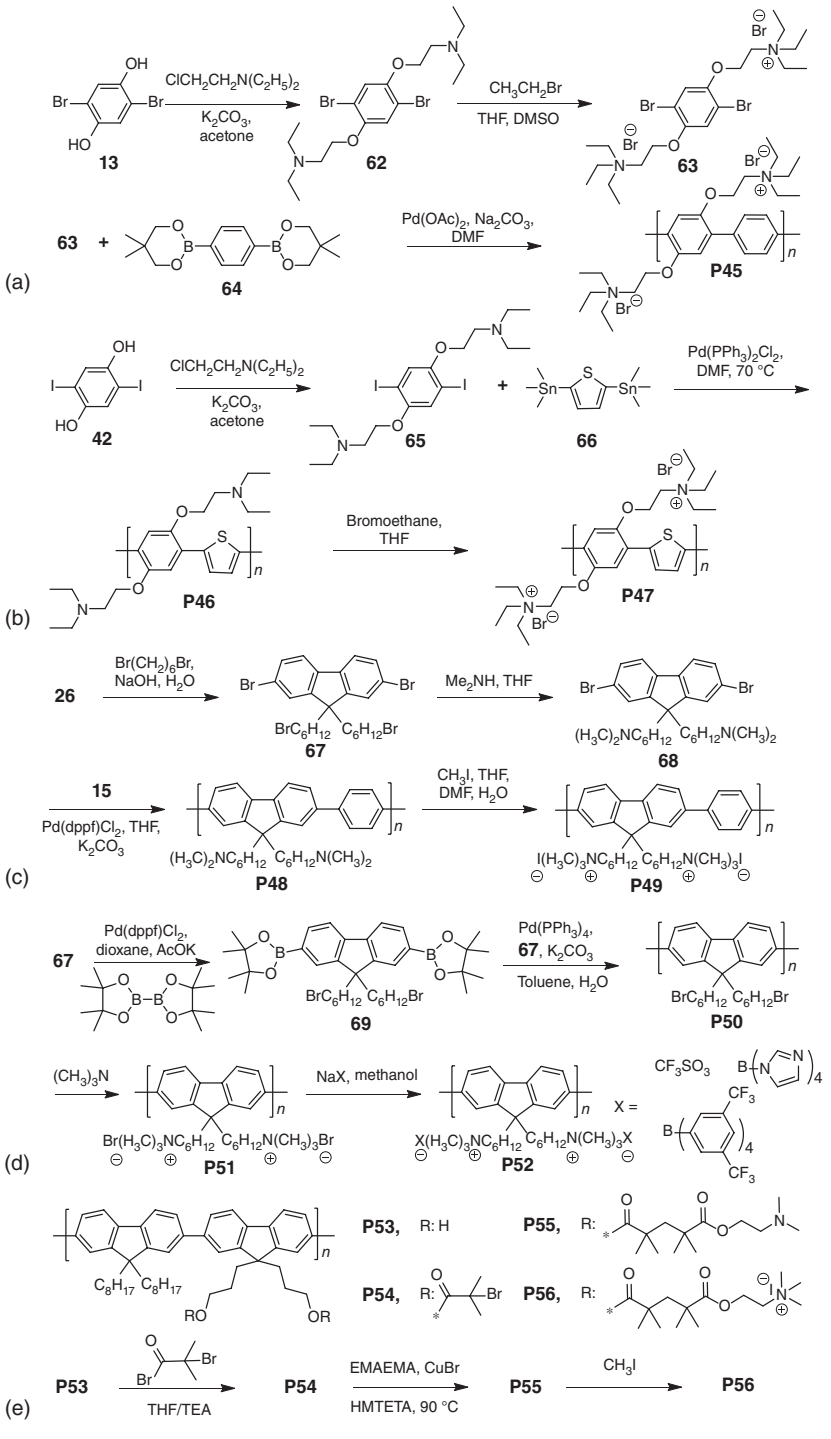


Scheme 1.13 Synthesis of ammonium polythiophenes **P39**, **P41**, and **P44**.

To synthesize single-bonded ammonium CPEs, a direct polymerization strategy was used to synthesize **P45** using Suzuki polymerization (Scheme 1.14a) [50]. Compound **62** was synthesized through Williamson etherification of **13** by refluxing in acetone with 2-chloroethyldiethylamine and K_2CO_3. The key monomer **63** was obtained by quaternization of **62** with bromoethane. Subsequent Suzuki polymerization between **63** and 1,4-bis(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzene **64** afforded the ammonium **P45**.

The same group also employed Stille coupling reaction to synthesize thiophene-containing poly(*p*-phenylene) **P47** [51]. As shown in Scheme 1.14b, the key monomer **65** was synthesized using a similar procedure to **62**. It is noted that the diiodo-substituted monomer was chosen over a dibromo-substituted monomer due to its higher reactivity in Pd-catalyzed coupling reaction. Stille coupling reaction between **65** and 2,5-bis(trimethylstannyl)thiophene **66** led to the neutral polymer **P46**, which was treated with bromoethane to afford ammonium poly(*p*-phenylene) **P47**.

In 2002, Bazan's group reported an ammonium polyfluorene via postpolymerization method. **P49** was obtained by the sequence of reactions shown in Scheme 1.14c [52]. Deprotonation of 2,7-dibromofluorene **26** with NaOH in a



Scheme 1.14 Synthesis of ammonium CPEs **P45**, **P47**, **P49**, **P51**, **P52**, and **P56** with single-bonded backbones.

two-phase mixture of water and 1,6-dibromohexane provided 2,7-dibromo-9,9-bis(6′-bromohexyl)fluorene **67**, which was treated with dimethylamine to give 2,7-dibromo-9,9-bis(6′-(*N*,*N*-dimethylamino)hexyl)fluorene **68**. Direct Suzuki polymerization between **68** and 1,4-phenyldiboronic acid **15** at a stoichiometric ratio yielded the neutral precursor polymer **P48**. Quaternization of **P48** with iodomethane in a mixture of THF/DMF/water led to the alternating ammonium **P49** in a moderate yield.

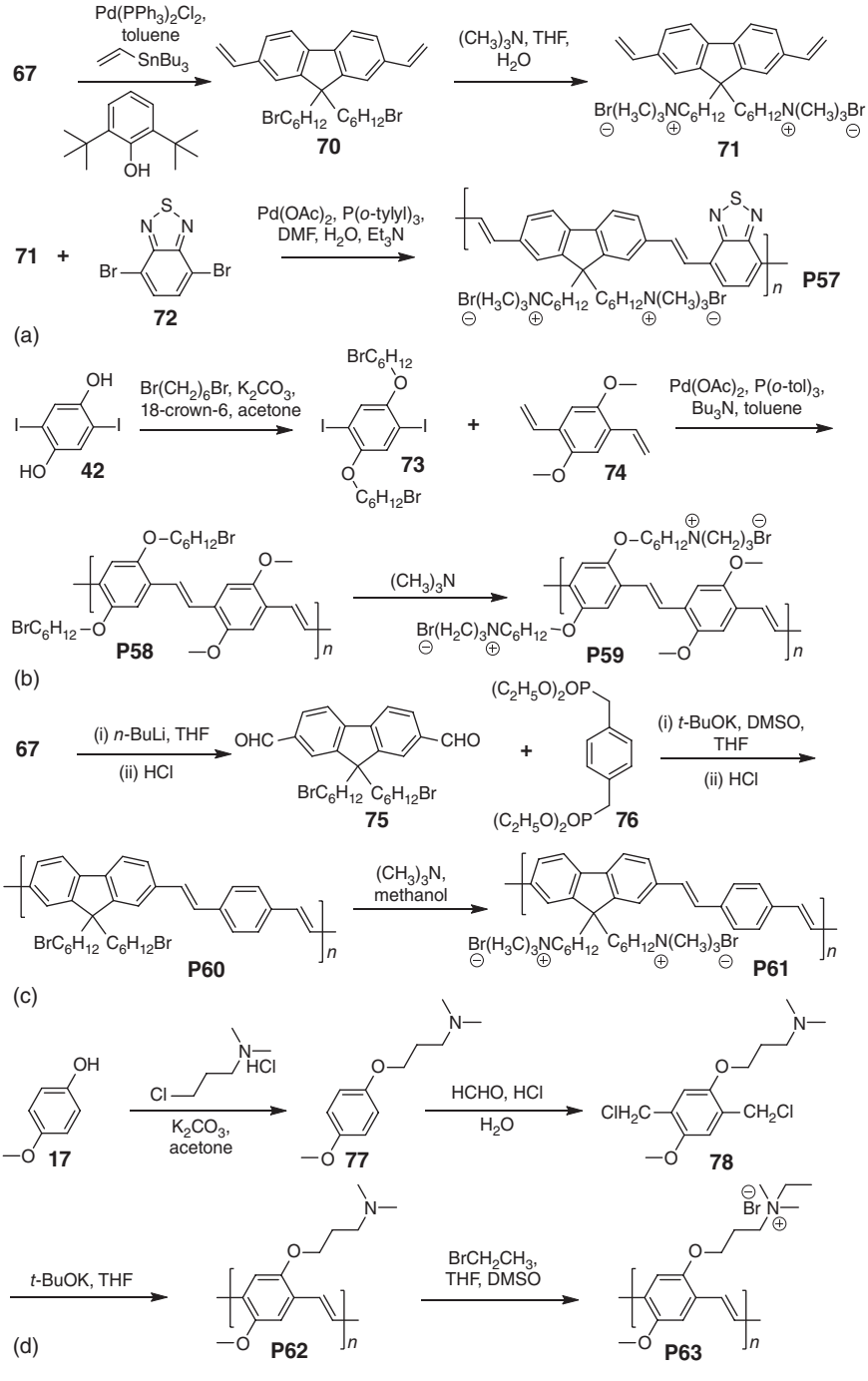
The same group also used another postpolymerization method to synthesize homopolyfluorene with different counterions [53]. As shown in Scheme 1.14d, Suzuki–Miyauri reaction between **67** and bis(pinacolato)diboron in the presence of AcOK and $Pd(dppf)_2Cl_2$ in anhydrous dioxane afforded the fluorene boronate ester **69**. The Suzuki polymerization between **67** and **69** using $Pd(PPh_3)_4$ as catalyst and Na_2CO_3 as base in a two-phase mixture of toluene/water yielded the neutral precursor polymer **P50**. Owing to the highly efficient reaction between alkyl bromide and the trimethylamine, subsequent reaction between **P50** and trimethylamine resulted in **P51** with more than 95% degree of quaternization. Upon treatment of **P51** with an excess of different salts, **P52** was obtained with different counter ions.

In 2010, Huang's group successfully synthesized a water-soluble grafted CPE **P56** via atom transfer radical polymerization (ATRP) for the first time (Scheme 1.14e) [54]. Starting from **P53,** which was synthesized by Suzuki polymerization, reaction with 2-bromoisobutyryl bromide afforded the macroinitiator **P54**, which was used to prepare **P55** via the ATRP approach. Subsequent reaction with methyl iodide gave **P56**. The brush-like side-chain architecture of polyfluorenes **P56** endows it with an extremely high ammonium ion density, consequently giving rise to high water solubility ($28\,mg\,ml^{-1}$) and high quantum efficiency (52%).

To synthesize ammonium CPEs with double-bonded backbones, Heck coupling reaction was used. As shown in Scheme 1.15a, 9,9-bis(6′-bromohexyl)-2,7-divinylfluorene **70** was obtained by heating the mixture of **67** and tributylvinyltin in toluene using $Pd(PPh_3)_2Cl_2$ as catalyst and 2,6-di-*tert*-butylphenol as the inhibitor. Subsequent quaternization of **70** with trimethylamine in a mixture of THF/water afforded the monomer 9,9-bis(6′-(*N*,*N*,*N*-trimethylammonium) hexyl)-2,7-divinylfluorene dibromide **71**. Finally, the ammonium **P57** was obtained via $Pd(OAc)_2/P(o\text{-tolyl})_3$-catalyzed direct Heck coupling polymerization of **71** and 4,7-dibromo-2,1,3-benzothiazole **72** in a solvent mixture containing DMF/H_2O/TEA (v/v/v = 2/1/2) [55].

A postpolymerization method was also reported by Wang's group using Heck coupling reaction. As shown in Scheme 1.15b, alkylation of **42** with 1,6-dibromohexane and K_2CO_3 in acetone afforded **73**. Heck reaction between **73** and the divinyl monomer **74** led to the neutral polymer **P58**. The presence of the active bromide atom of **P58** allows the facile synthesis of **P59** through quaternization with trimethylamine [56].

Another approach to synthesize poly(fluorene vinylene)s is through the Witting polymerization method [57]. As shown in Scheme 1.15c, the key monomer 2,7-dicarbaldehyde-9,9-bis(6′-bromohexyl)fluorene **75**, was obtained by treatment of **67** with BuLi followed by the addition of anhydrous DMF. The Witting–Horner condensation reaction between **75** and 1,4-bis(diethylphosphinatylmethyl)phenylene

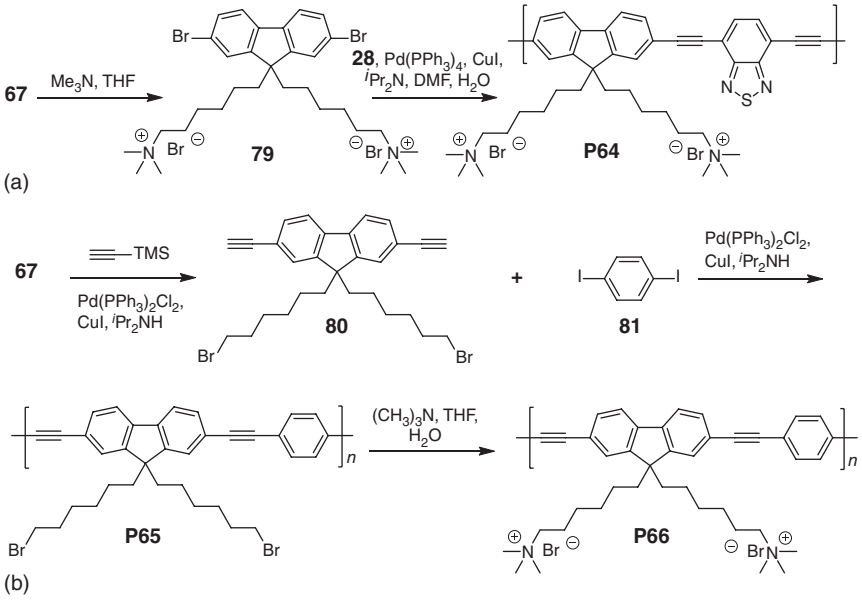


Scheme 1.15 Synthesis of ammonium CPEs **P57**, **P59**, **P61**, and **P63** with double-bonded backbones.

76 in dry THF in the presence of *t*-BuOK led to the precursor **P60**, which was converted to ammonium **P61** via quaternization with trimethylamine in chloroform/methanol.

Gilch coupling reaction was also employed by Shen's group to synthesize ammonium poly(*p*-phenylene vinylene)s [58]. As shown in Scheme 1.15d, 3-(4-methoxyphenoxy)-*N*,*N*-dimethylpropan-1-amine **77** was synthesized through Williamson etherification of **17** by refluxing in acetone with 3-chloropropyldimethylamine and K_2CO_3. Chlorination of **77** with a 37% formalin solution in concentrated hydrochloric acid resulted in the key monomer **78**. Gilch reaction of **78** was performed in THF in the presence of *t*-BuOK to yield the precursor polymer **P62**, which was treated with bromoethane in a mixture of THF/DMSO to give the ammonium poly(*p*-phenylene vinylene) **P63**.

Sonogashira reaction was often used to prepare ammonium CPEs with triple-bonded backbones. As shown in Scheme 1.16a, quaternization of **67** with trimethylamine in THF/water yielded the ammonium monomer **79**, which was directly copolymerized with 4,7-diethynyl-2,1,3-benzothiadiazole **28** to yield **P64** via Sonogashira reaction using $Pd(PPh_3)_4$/CuI as catalysts in a mixed solution of DMF/H_2O/diisopropylamine (v/v/v = 2/1/1.5) [32].

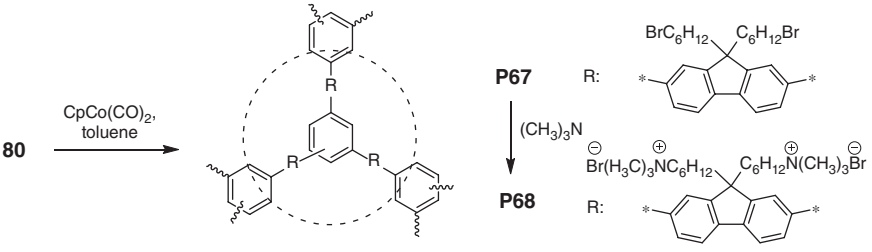


Scheme 1.16 Synthesis of ammonium polyfluorenes **P64** and **P66** with triple-bonded backbones.

Another approach to synthesize poly(fluorene ethynylene)s is via postpolymerization. As shown in Scheme 1.16b, the reaction between **67** and trimethylsilyl acetylene using $Pd(PPh_3)_2Cl_2$/CuI as catalysts followed by trimethylsilyl deprotection in a basic solution led to 9,9-bis(6-bromohexyl)-2,7-diethynylfluorene **80**. The ethynyl and bromide groups were kept intact during the reactions. **80** was copolymerized with 1,4-diiodobenzene **81**

through $Pd(PPh_3)_4$/CuI-catalyzed Sonogashira reaction in a mixture of toluene/diisopropylamine (v/v = 2/1) to yield **P65**. Quaternization of **P65** with trimethylamine afforded the ammonium **P66** [59].

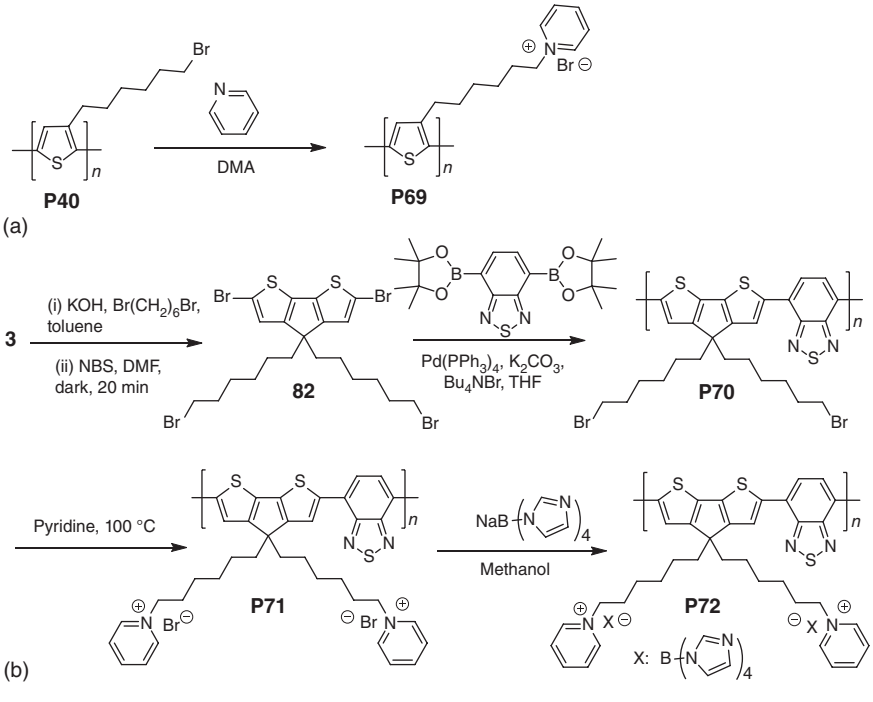
Apart from the above-discussed linear cationic CPEs, Liu's group also reported hyperbranched ammonium polyfluorenes. For example, **P67** was synthesized through 2 steps from **80**. As shown in Scheme 1.17, $CpCo(CO)_2$- catalyzed homopolycyclotrimerization of **80** with UV irradiation led to hyperbranched neutral **P67**, which was converted to **P68** after treatment with trimethylamine [60].



Scheme 1.17 Synthesis of hyperbranched CPE **P68**.

1.2.2.2 Pyridinium CPEs

Pyridinium CPEs are generally obtained through quaternization between pyridine and active halogen atoms (e.g., Br). As shown in Scheme 1.18a, Fukuhara and Inoue reported pyridinium polythiophene **P69** through treatment of **P40**

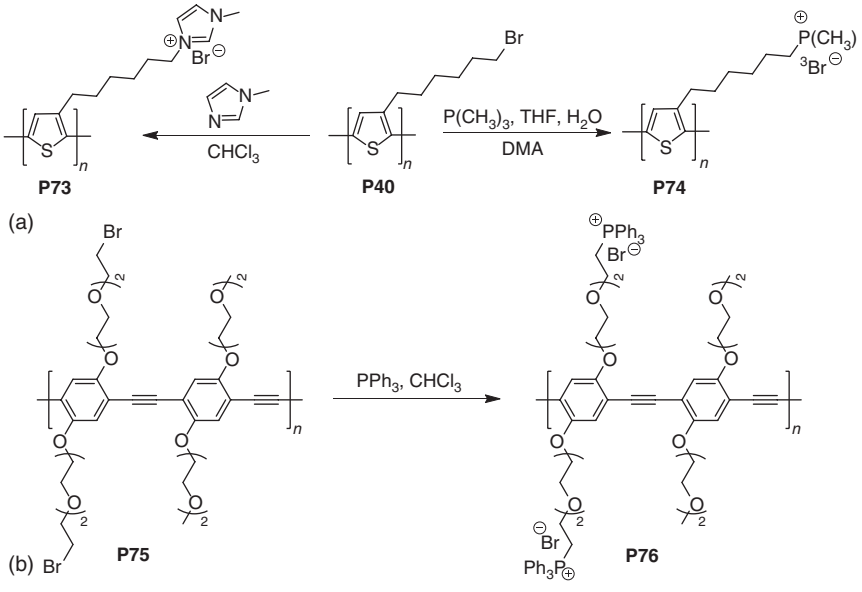


Scheme 1.18 Synthesis of pyridinium CPEs **P69**, **P71**, and **P72**.

with pyridine in *N*,*N*-dimethylacetamide (DMA) [61]. Bazan's group also reported pyridinium CPEs **P71** and **P72** with narrow band gap (Scheme 1.18b) [62]. Starting from **3**, alkylation with 1,6-dibromohexane and KOH followed by bromination of the intermediate using *N*-bromosuccinimide yielded the dibromide-substituted cyclopentadithiophene **82**. Suzuki polymerization between **82** and the bis-boronate ester of benzothiadiazole generated the neutral precursor polymer **P70**. Quaternization of **P70** with pyridine led to the pyridinium **P71**. In addition, bromide counter ion in **P71** has also been further exchanged with the large tetrakis(1-imidazolyl)borate (BIm_4^-) to yield **P72**.

1.2.2.3 Phosphonium CPEs

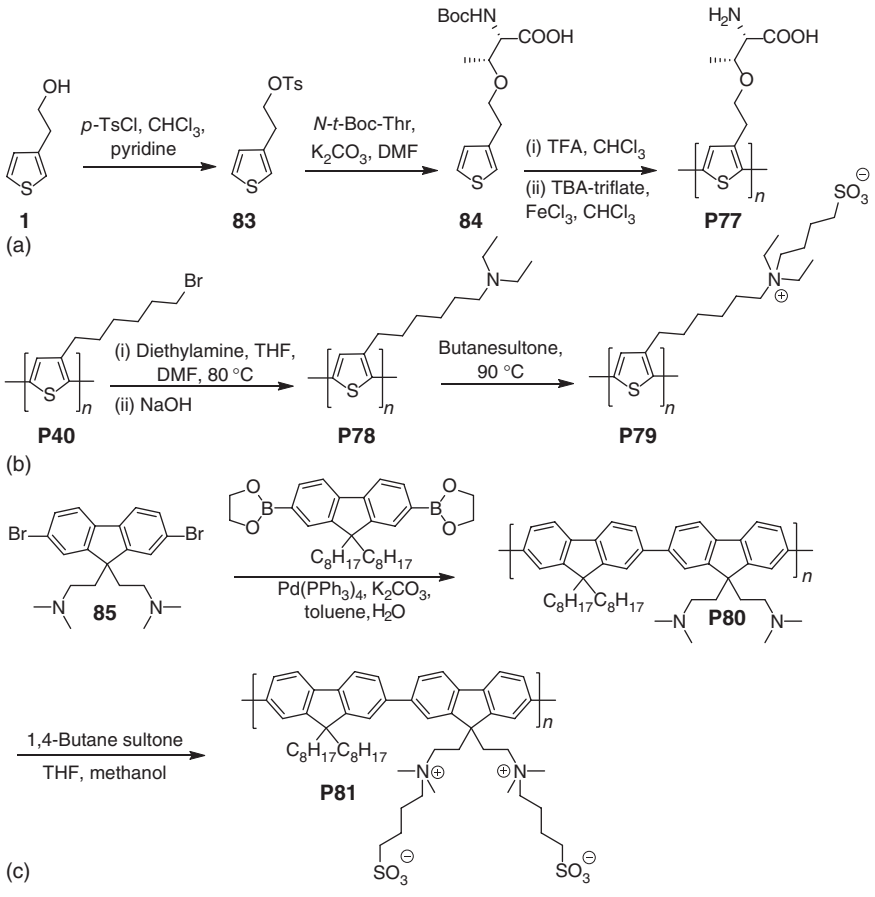
In addition to the ammonium and pyridinium salt, cationic CPEs with other types of charges (e.g., phosphonium) have also been reported. As shown in Scheme 1.19a, quaternization of the neutral polymer **P40** with 1-methyl-imidazole or trimethylphosphine led to **P73** or **P74**, respectively [63]. The obtained CPEs are insoluble in common organic solvents (e.g., THF, chloroform, dichloromethane, and toluene) but readily soluble in water. Using a similar strategy, **P75** was treated with PPh_3 to yield the corresponding phosphonium **P76** (Scheme 1.19b) [64].



Scheme 1.19 Synthesis of cationic CPEs **P73**, **P74,** and **P76**.

1.2.3 Zwitterionic CPEs

Different from anionic and cationic CPEs, zwitterionic CPEs contain side groups with anionic and cationic functionalities that are covalently linked with each other. Zwitterionic polythiophenes have been synthesized by Konradsson's



Scheme 1.20 Synthesis of zwitterionic CPEs **P77**, **P79**, and **P81**.

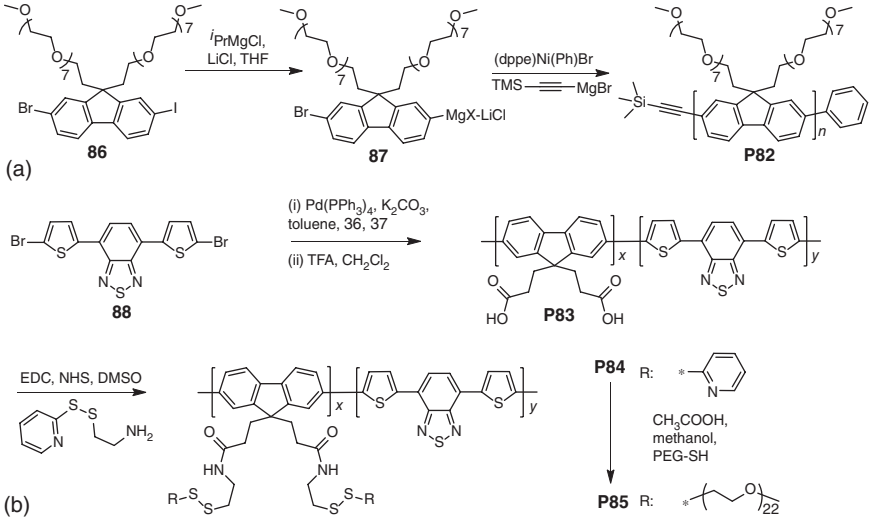
group. As shown in Scheme 1.20a, **1** was tosylated with *p*-TsCl in the presence of pyridine to give a thiophene derivative **83**. Alkylation of **83** with a Boc-protected amino acid, *N-t*-Boc-Thr, yielded the key monomer **84**. After Boc-deprotection with trifluoroacetic acid, $FeCl_3$-catalyzed oxidative polymerization of the product gave the zwitterionic polythiophene **P77** [65].

Another type of zwitterionic polythiophene **P79** was also synthesized. As shown in Scheme 1.20b, the precursor polymer **P40** first reacted with diethylamine in a mixture of THF/DMF to give **P78**, which was converted to **P79** using 1,4-butane sultone as the quaternization agent and solvent [66].

In 2011, Huck's group reported a zwitterionic polyfluorene as the charge injection layer for high-performance polymer light-emitting diodes [67]. As shown in Scheme 1.20c, the Pd-mediated Suzuki polymerization between **85** and di-boronate ester fluorene yielded a neutral polymer **P80**. After quaternization of **P80** using 1,4-butane sultone in a THF/methanol solvent mixture, **P81** was obtained with near 100% conversion of the tertiary amines into sulfobetaine zwitterionic groups [67].

1.3 Neutral WSCPs

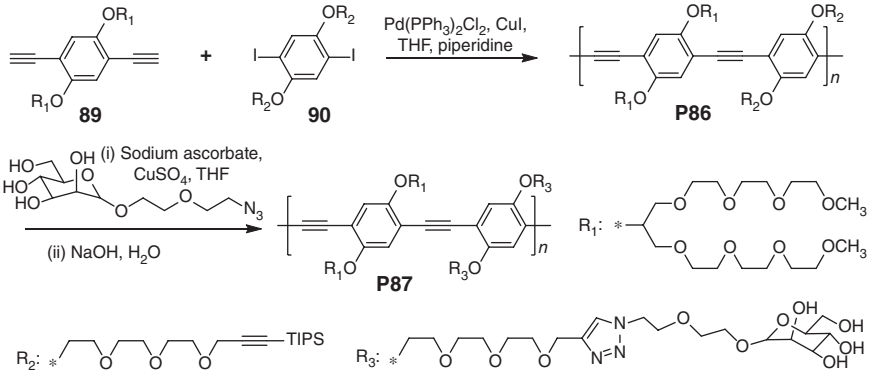
In the previous section, we have described the various approaches to synthesize CPEs with different charge signs. In this section, we will briefly discuss the synthesis of neutral WSCPs. Neutral WSCPs contain neutral polar functionalities, for example, oligo(ethylene glycol) segments, that compensate for the hydrophobic nature of the conjugated backbones. Neutral WSCPs often exhibit high fluorescence quantum yield, good water solubility, and strong resistance to nonspecific interactions. As shown in Scheme 1.21a, Bazan's group reported the synthesis of a neutral WSCP **P82** using the complex *cis*-(bromo)(phenyl)[1,2-bis(diphenylphosphino)ethane]nickel as the initiator and **86** as the monomer in the presence of isopropylmagnesium chloride–lithium chloride, followed by quenching the polymerization with [2-(trimethylsilyl)ethynyl]magnesium bromide [11]. Owing to the incorporation of octakis(ethylene glycol) side chains, **P82** exhibited good solubility in water and high-fluorescence quantum yield (80%). In addition, each **P82** chain possesses one terminal silylacetylene group, which allowed further conjugation with functional components via click reaction. This provides a versatile strategy to fabricate ideal fluorescent probes for biosensing applications.



Scheme 1.21 Synthesis of neutral WSCPs **P82** and **P85**.

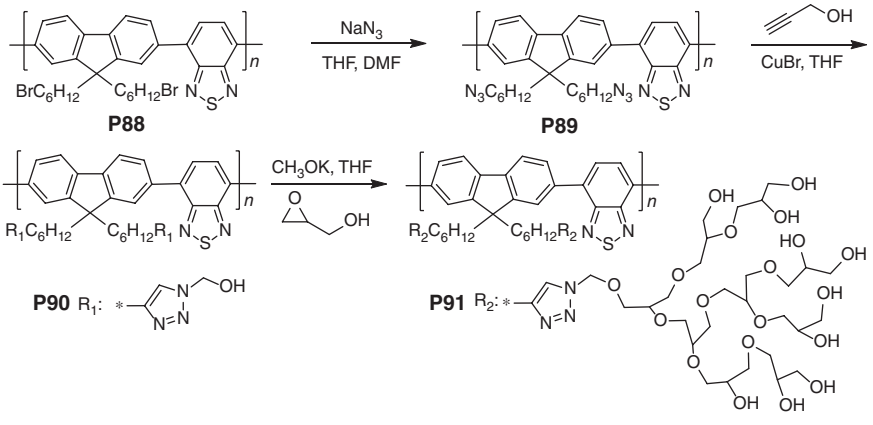
Meanwhile, Huang's group reported a postpolymerization method to synthesize WSCP **P85** with oligo(ethylene glycol) side chains [68]. As shown in Scheme 1.21b, Suzuki polymerization of monomers **36**, **37**, and **88** followed by deprotection of ester groups with trifluoroacetic acid yielded **P83**. Subsequently, **P83** was modified with 2-(pyridyldithio)ethylamine through amidation to yield **P84**, which was converted to **P85** through the replacement of pyridine units with PEG-SH chains.

Bunz's group also used Sonogashira reaction to prepare neutral water soluble poly(*p*-phenylene ethynylene) **P87** [69]. As shown in Scheme 1.22, Pd-catalyzed Sonogashira coupling between **89** and **90** furnished the neutral polymer **P86**, which was deprotected *in situ* and subjected to a cupper catalyzed 1,3-dipolar cycloaddition with azide-functionalized sugar resulting in **P87**. Because of the presence of polar side chains (sugar and oligo(ethylene glycol)), **P87** can be freely dissolved in water.



Scheme 1.22 Synthesis of neutral WSCP **P87**.

Liu's group developed another strategy to prepare WSCPs using hyperbranched polyglycerol as brush due to its good biocompatibility [70]. This method involves the combination of "grafting from" strategy and living ring-opening polymerization technique. As shown in Scheme 1.23, Starting from **P88**, reaction with NaN_3 in a mixture of THF/DMF afforded **P89**. Subsequent click reaction with propargyl alcohol led to the macroinitiator **P90**, which was used to incorporate hyperbranched polyglycerol as brush via ring-opening polymerization technique, yielding **P91** with good water

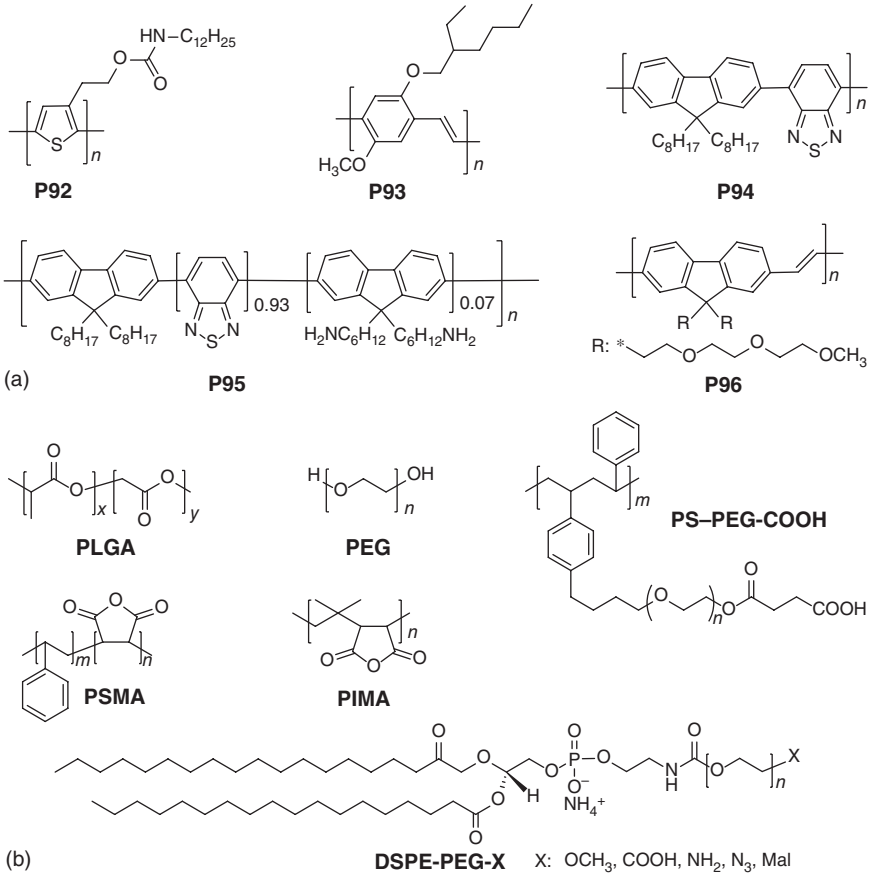


Scheme 1.23 Synthesis of neutral WSCP **P91** with HPG brush.

solubility. In addition, the same group also reported many CPs with poly(ethylene glycol) brush through click reaction between poly(ethylene glycol) and neutral precursor CP [71–73].

1.4 Fabrication of CPNPs

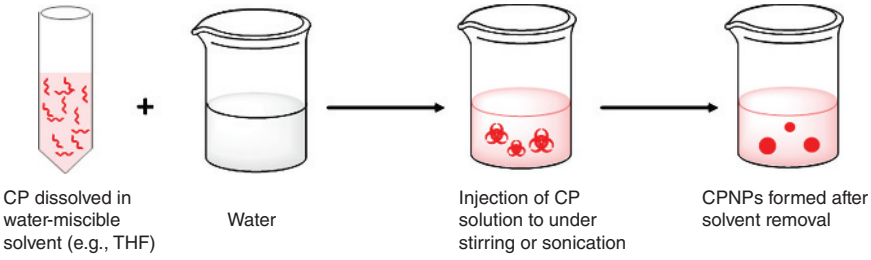
Compared to the chemical synthesis of CPs, preparation of CPNPs directly from organic solvent-soluble CPs represents another strategy to bring CPs into aqueous media. To simplify the discussion, all the NPs prepared from organic soluble CPs are termed as CPNPs in this chapter. The most widely used approaches for CPNPs synthesis include reprecipitation, miniemulsion, and nanoprecipitation [9, 12, 74]. In the following section, we choose some specific examples to illustrate each method by discussing their advantages and disadvantages. Scheme 1.24 shows the chemical structures of representative CPs and amphiphilic materials used for CPNP preparation.



Scheme 1.24 Representative chemical structures of CPs and amphiphilic materials used for CPNP preparation.

1.4.1 Reprecipitation

Reprecipitation method was first applied to CPs by Masuhara's group [75]. In a typical reprecipitation method schematically shown in Scheme 1.25, a small amount of hydrophobic CP is dissolved in a water-miscible solvent (e.g., THF). Next, the organic solution is rapidly injected into excess water under vigorous stirring or sonication. The large solubility discrepancy of the CP in two solvents as well as the hydrophobic interactions between polymer chains induces the nanoparticle formation during the organic solvent evaporation. This method does not involve the use of any additives such as surfactants. Using this method, Masuhara's group prepared **P92** NPs with sizes ranging from 40 to 420 nm by injecting a solution of **P92** in THF into vigorously stirred distilled water [75]. The size can be tuned by changing the concentration of **P92** in THF or the temperatures of water (e.g., from 20 to 80 °C). The lower concentration provided the smaller size. The obtained CPNPs exhibited size-dependent spectroscopic properties.

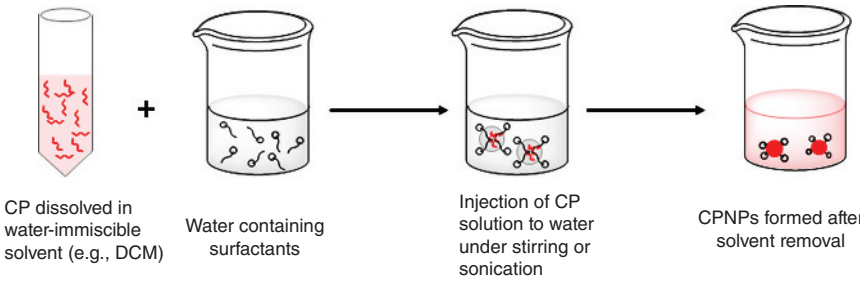


Scheme 1.25 Schematic illustration of the preparation of CPNPs using reprecipitation method.

Subsequently, McNeill's group reported a modified procedure to prepare CPNPs with the sizes ranging from 5 to 30 nm [76]. In this specific case, 2 ml of **P93** in THF with a very low concentration (0.005 wt%) was added quickly to 8 ml of deionized water under sonication conditions. After evaporation of the THF under vacuum, filtration with a 0.2 μm membrane filter was performed to afford **P93** NPs with major sizes between 5 and 10 nm. **P92** and **P93** NPs exhibit spherical shape. This method is applicable to a wide range of CPs that are soluble in water-miscible organic solvents. In addition, it is possible to adjust the particle size via tuning the CP concentration or selecting CPs with appropriate molecular weight. However, due to the inherent hydrophobic nature of CP, the obtained CPNPs tend to aggregate into large sized particles, thus precipitating from water. Therefore, these CPNPs were only obtained with a very low concentration (e.g., 0.005%), and could not be stored for a long period.

1.4.2 Miniemulsion

Miniemulsions are specially formulated heterophase systems consisting of stable nanodroplets in a continuous phase (e.g., water). In a typical miniemulsion procedure illustrated in Scheme 1.26, the CP dissolved in a water-immiscible organic



Scheme 1.26 Schematic illustration of the preparation of CPNPs using miniemulsion method.

solvent (e.g., dichloromethane, chloroform) is added to an aqueous solution containing an appropriate surfactant. The mixture is treated with ultrasonication to form stable miniemulsions containing small organic droplets of the CP solution. After solvent evaporation, the droplets collapse to form stable water-dispersible CPNPs. Compared to the reprecipitation method, the miniemulsion method employs surfactant to form and stabilize droplets. The sizes of formed CPNPs via this method vary from 2 to 500 nm, depending on the nature and concentration of the polymer and surfactant used.

Using the miniemulsion method, Liu's group reported a generic strategy to prepare a series of multicolor CPNPs with emission spanning from 400 to 700 nm, using Food and Drug Administration (FDA)-approved poly(DL-lactide-*co*-glycolide) (PLGA) as the encapsulation matrix, and poly(vinyl alcohol) (PVA) as the emulsifier [77]. Herein, we still choose **P93** as an example. In this case, a dichloromethane solution containing **P93** and PLGA was poured into an aqueous solution containing PVA. After sonicating the mixture, the resulting emulsion was stirred at room temperature to remove the solvent. Then, **P93** NPs were obtained after careful washing and centrifugation to remove the emulsifier and free **P93** molecules. Although the resultant CPNPs show good colloidal stability in water, their sizes are in the range of 240–270 nm and the encapsulation efficiencies are relatively low (~45%).

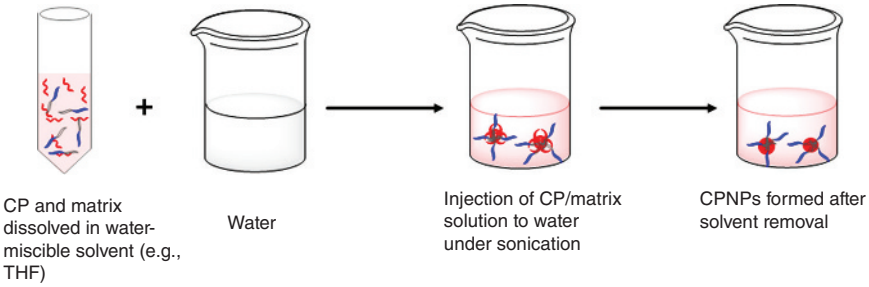
Meanwhile, Green's group reported a simple strategy to synthesize a series of PEG encapsulated CPNPs with emission spanning from blue to green, yellow, and red [78]. Using **P94** as an example, the dilute **P94** solution in dichloromethane (40 ppm by weight) was added dropwise to an aqueous PEG solution while stirring vigorously with ultrasonication. After sequential evaporation of the remaining dichloromethane, filtration with filter paper, and centrifugation, the **P94** NPs were obtained with mean diameters ranging from 2 to 3 nm and a quantum yield of ~12%. The presence of PEG is essential to stabilize the formed NPs, as the **P94** NPs prepared without PEG are not stable. However, a considerable amount of fluorescence quenching was observed in the small CPNPs.

Compared to the straightforward strategy to synthesize CPNPs from preprepared CPs, another strategy to synthesize CPNPs is miniemulsion polymerization. This method generally employs water-tolerance reactions, such as Sonogashira, Suzuki, and $FeCl_3$-catalyzed oxidative polymerization reactions.

Generally, the appropriate monomers are dispersed in surfactant-containing aqueous solution under vigorous stirring to form stable nanodroplets. During this process, the hydrophobic monomers are embedded in the interior. After addition of appropriate catalyst, polymerization and nanoparticle formation are achieved concurrently. This strategy will be discussed in detail in the next chapter.

1.4.3 Nanoprecipitation

Nanoprecipitation is a modification of the reprecipitation method, and has become the most commonly used approach for the preparation of CPNPs. In a typical procedure as shown in Scheme 1.27, CP and amphiphilic encapsulation matrix are first dissolved well in a water-miscible organic solvent (e.g., THF). The resulting solution is then quickly added into a water phase under sonication to form a stable nanoparticle dispersion. After organic solvent removal, the matrix encapsulated CPNPs were obtained. During nanoparticle formation, the hydrophobic components of the matrix and CPs are likely embedded into the NP core while the hydrophilic segments of the matrix are oriented to the aqueous environment. The NPs prepared by this method show better colloidal stability than those obtained from reprecipitation. The particle size and optical and surface chemical properties of the CP NPs can be adjusted by changing the CPs, amphiphilic matrix, and other related parameters.



Scheme 1.27 Schematic illustration of the preparation of CPNPs using nanoprecipitation method.

In 2010, Chiu's group synthesized CPNPs based on **P94** via the nanoprecipitation method using a functional, amphiphilic, comb-like poly(styrene-*g*-ethylene oxide) (PS–PEG-COOH) as an encapsulation matrix [79]. PS–PEG-COOH consists of a hydrophobic polystyrene backbone and several hydrophilic PEG side chains terminated with carboxylic acid. Briefly, a precursor THF solution with a constant **P94** concentration and PS–PEG-COOH/**P94** fractions ranging from 0 to 20 wt% was quickly added to water in a bath sonicator to afford carboxylic-functionalized CPNPs of **P94**. During NP formation, the hydrophobic polystyrene segment and **P94** are most likely embedded inside the NPs, while the hydrophilic PEG chains and carboxylic acid groups extend outside into the aqueous environment. Moreover, the PEG chains not only function as a biocompatible layer to minimize nonspecific absorption, but also provide a steric barrier against nanoparticle aggregation and

carboxyl groups for further conjugation. The obtained CPNPs were determined by dynamic light scattering (DLS) to have an average diameter of 15 nm and a quantum yield of 30% in water. Replacement of PS–PEG-COOH with unfunctionalized PS–PEG did not cause any noticeable effects on particle size and morphology. In addition, increase of the concentration of **P94** in THF solution produced larger sized NPs. The absorption and emission of NPs based on **P94** show size-independent features.

Later on, the same group reported another strategy to prepare **P94** NPs using poly(styrene-*co*-maleic anhydride) (PSMA) as an encapsulation matrix [80]. The preparation procedure is similar to that of PS–PEG-COOH encapsulated **P94** NPs. During NP formation, the hydrophobic polystyrene units of PSMA and **P94** were anchored inside the particles while the hydrophilic maleic anhydride units were most likely localized on the NP surface and hydrolyzed in the aqueous environment to produce carboxylate groups on the NP surface for further conjugations. The obtained **P94** NPs show comparable size and fluorescence quantum yield to PS–PEG-COOH encapsulated **P94** NPs.

The nanoprecipitation method discussed above has two drawbacks: (i) The matrices are likely to dissociate from the formed NPs and (ii) it is difficult to achieve CPNPs with sizes smaller than 10 nm. To solve these problems, Chiu's group further developed a cross-linking strategy to synthesize stable CPNPs based on **P95**. In this case, poly(isobutylene-*alt*-maleic anhydride) (PIMA) was chosen as matrix and cross-linker [81]. PIMA, which has multiple reactive units, first reacts with the amine-functionalized **P95** to form covalent cross-links. Then, the resultant product dissolved in THF was injected quickly into Milli-Q water under sonication to afford **P95** NPs with carboxylic groups on the particle surface. As compared to the **P94** NPs, **P95** NPs showed lower fluorescence quantum yield (21% vs 30%) but better colloidal stability.

PEG-functionalized lipid is another matrix commonly used for CP encapsulation. The first lipid-PEG encapsulated **P94** NPs were reported by Christensen's group in 2011 [82]. They synthesized a series of lipid-PEG (PEG $M_r = 2000$, 1000, 500) with carboxy, biotin, or methoxy end groups. To synthesize **P94** NPs, a dilute solution of **P94** in THF was rapidly added to a ninefold volume of water containing lipid-PEG under continuous mild sonication. During this process, the aliphatic side chains on the **P94** interact with the hydrophobic lipid-PEG tail and are inserted into the NP core, while the hydrophilic PEG groups protrudes out into the aqueous media. The DLS diameters of the formed NPs are 20–30 nm, and are insensitive to the variation in the PEG length and end groups. However, the obtained **P94** NPs have low concentration and moderate fluorescence quantum yield (~18%). Subsequently, Liu's group modified this method to synthesize CPNPs based on a wide variety of preprepared CPs for bioapplications using the matrix of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE)-PEG_{2000} with different terminal functional groups, such as carboxylic acid, amine, azide, and maleimide [83].

Apart from PS- and lipid-derivatives, some proteins or peptides were also used as matrix to synthesize CPNPs via nanoprecipitation [84, 85]. For example, Liu's group used bovine serum albumin (BSA) as a polymer matrix to prepare **P96** NPs due to its good biocompatibility and nontoxicity [84]. The NP synthesis

started with the addition of a THF solution containing **P96** into a BSA aqueous solution under sonication, which was followed by cross-linking with glutaraldehyde and THF evaporation. The formed **P96** NPs exhibit DLS in the size of ~160 nm and carboxylic groups on the surface.

Encapsulation of CPs with silica shell is also an effective approach to synthesize CPNPs. Liu's group first reported on the synthesis of SiO_2@CP@SiO_2 NPs at room temperature without specific CP design or silane modification [86]. Using **P94** as an example, the THF solution of **P94** was first poured into a mixture of ethanol/water (v/v = 9/1) under sonication. As THF evaporates, **P94** formed small dots. Subsequently, a silica precursor, tetraethyl orthosilicate (TEOS) as well as ammonia was injected into the resulting mixture with vigorous stirring. After 12 h, an amine-functionalized silica precursor, 3-aminopropyl triethoxysilane (APTES), was added to modify the obtained SiO_2@**P94**@SiO_2 NPs with amine groups on surface. It was noted that the resulting NPs were ~70 nm in diameter with a quantum yield of 27% in water. The authors used four different CPs to prepare silica encapsulated NPs in their study, demonstrating that this method can be applied to any CPs that are soluble in organic solvents.

1.5 Conclusion

This chapter describes the commonly used strategies to bring CPs into aqueous media for biological applications. The water-soluble or water-dispersible CPs can be realized via either chemical modification of the conjugated backbones and side chains for CPEs and neutral WSCPs or by preparation of CP-based CPNPs. According to their chemical structures, the synthesis of CPEs and neutral WSCPs involves two aspects: construction of conjugated backbones and incorporation of charged or polar side chains. The conjugated backbones have been built through well-established polymerization methods, which are typically catalyzed with organometallic complexes or bases, while the functional side chains can be obtained through either direct polymerization or postpolymerization methods. For the preparation of CPNPs, three strategies are generally employed to encapsulate organic-soluble CPs and bring them in aqueous media, namely, reprecipitation, miniemulsion, and nanoprecipitation. Despite the great success in bringing organic-soluble CPs into water in the past decades, there is much to be done to understand how molecular packing and polymer aggregation affect their optical properties and how CP structures and performance can be optimized in order to meet the requirements for existing as well as new applications.

References

1 Pei, Q., Yu, G., Zhang, C., Yang, Y., and Heeger, A.J. (1995) *Science*, **269**, 1086.
2 Friend, R., Gymer, R., Holmes, A., Burroughes, J., Marks, R., Taliani, C., Bradley, D., Dos Santos, D., Bredas, J., and Lögdlund, M. (1999) *Nature*, **397**, 121–128.

3 Grimsdale, A.C., Leok Chan, K., Martin, R.E., Jokisz, P.G., and Holmes, A.B. (2009) *Chem. Rev.*, **109**, 897–1091.
4 Cheng, Y.-J., Yang, S.-H., and Hsu, C.-S. (2009) *Chem. Rev.*, **109**, 5868–5923.
5 Huang, F., Wu, H., and Cao, Y. (2010) *Chem. Soc. Rev.*, **39**, 2500–2521.
6 Liu, B. and Bazan, G.C. (2004) *Chem. Mater.*, **16**, 4467–4476.
7 Feng, L., Zhu, C., Yuan, H., Liu, L., Lv, F., and Wang, S. (2013) *Chem. Soc. Rev.*, **42**, 6620–6633.
8 Zhu, C.L., Liu, L.B., Yang, Q., Lv, F.T., and Wang, S. (2012) *Chem. Rev.*, **112**, 4687–4735.
9 Li, K. and Liu, B. (2012) *J. Mater. Chem.*, **22**, 1257–1264.
10 Pinto, M.R. and Schanze, K.S. (2002) *Synthesis*, **2002**, 1293–1309.
11 Traina, C.A., Bakus, R.C. 2nd, and Bazan, G.C. (2011) *J. Am. Chem. Soc.*, **133**, 12600–12607.
12 Tuncel, D. and Demir, H.V. (2010) *Nanoscale*, **2**, 484–494.
13 Pu, K.-Y., Shi, J., Cai, L., Li, K., and Liu, B. (2011) *Biomacromolecules*, **12**, 2966–2974.
14 Patil, A., Ikenoue, Y., Wudl, F., and Heeger, A. (1987) *J. Am. Chem. Soc.*, **109**, 1858–1859.
15 Masuda, H. and Kaeriyama, K. (1992) *Die Makromol.Chem., Rapid Commun.*, **13**, 461–465.
16 Viinikanoja, A., Lukkari, J., Ääritalo, T., Laiho, T., and Kankare, J. (2003) *Langmuir*, **19**, 2768–2775.
17 Zotti, G., Zecchin, S., Schiavon, G., and Berlin, A. (2001) *Macromolecules*, **34**, 3889–3895.
18 Xue, C., Cai, F., and Liu, H. (2008) *Chem.– Eur. J.*, **14**, 1648–1653.
19 Rubio-Magnieto, J., Azene, E.G., Knoops, J., Knippenberg, S., Delcourt, C., Thomas, A., Richeter, S., Mehdi, A., Dubois, P., Lazzaroni, R., Beljonne, D., Clement, S., and Surin, M. (2015) *Soft Matter*, **11**, 6460–6471.
20 Zotti, G., Zecchin, S., Schiavon, G., Vercelli, B., Berlin, A., and Porzio, W. (2004) *Chem. Mater.*, **16**, 2091–2100.
21 Faid, K. and Leclerc, M. (1996) *Chem. Commun.*, 2761–2762.
22 Bellina, F., Carpita, A., and Rossi, R. (2004) *Synthesis*, **2004**, 2419–2440.
23 Rulkens, R., Schulze, M., and Wegner, G. (1994) *Macromol. Rapid Commun.*, **15**, 669–676.
24 Child, A.D. and Reynolds, J.R. (1994) *Macromolecules*, **27**, 1975–1977.
25 Shi, S. and Wudl, F. (1990) *Macromolecules*, **23**, 2119–2124.
26 Gu, Z., Shen, Q.D., Zhang, J., Yang, C.Z., and Bao, Y.J. (2006) *J. Appl. Polym. Sci.*, **100**, 2930–2936.
27 Gu, Z., Bao, Y.-J., Zhang, Y., Wang, M., and Shen, Q.-D. (2006) *Macromolecules*, **39**, 3125–3131.
28 Xie, B., Bagui, M., Guo, R., Li, K., Wang, Q., and Peng, Z. (2007) *J. Polym. Sci. A Polym. Chem.*, **45**, 5123–5135.
29 Zhang, W., Zhu, L., Qin, J., and Yang, C. (2011) *J. Phys. Chem. B*, **115**, 12059–12064.
30 Chinchilla, R. and Nájera, C. (2007) *Chem. Rev.*, **107**, 874–922.
31 Huang, F., Wang, X., Wang, D., Yang, W., and Cao, Y. (2005) *Polymer*, **46**, 12010–12015.

32 Pu, K.-Y. and Liu, B. (2010) *J. Phys. Chem. B*, **114**, 3077–3084.
33 Zhao, X., Pinto, M.R., Hardison, L.M., Mwaura, J., Müller, J., Jiang, H., Witker, D., Kleiman, V.D., Reynolds, J.R., and Schanze, K.S. (2006) *Macromolecules*, **39**, 6355–6366.
34 McCullough, R.D., Ewbank, P.C., and Loewe, R.S. (1997) *J. Am. Chem. Soc.*, **119**, 633–634.
35 Xing, C., Xu, Q., Tang, H., Liu, L., and Wang, S. (2009) *J. Am. Chem. Soc.*, **131**, 13117–13124.
36 Wallow, T.I. and Novak, B.M. (1991) *J. Am. Chem. Soc.*, **113**, 7411–7412.
37 Rau, I.U. and Rehahn, M. (1993) *Polymer*, **34**, 2889–2893.
38 Zhang, Y., Liu, B., and Cao, Y. (2008) *Chem.–Asian J.*, **3**, 739–745.
39 Peng, Z.H., Xu, B.B., Zhang, J.H., and Pan, Y.C. (1999) *Chem. Commun.*, 1855–1856.
40 Fujii, A., Sonoda, T., Fujisawa, T., Ootake, R., and Yoshino, K. (2001) *Synth. Met.*, **119**, 189–190.
41 Slaven, W.T. IV, Li, C.-J., Chen, Y.-P., John, V.T., and Rachakonda, S.H. (1999) *J. Macromol. Sci.—Pure Appl. Chem.*, **36**, 971–980.
42 Kim, I.B., Dunkhorst, A., Gilbert, J., and Bunz, U.H.F. (2005) *Macromolecules*, **38**, 4560–4562.
43 Qin, C., Wu, X., Gao, B., Tong, H., and Wang, L. (2009) *Macromolecules*, **42**, 5427–5429.
44 Qin, C., Wong, W.-Y., and Wang, L. (2010) *Macromolecules*, **44**, 483–489.
45 Pinto, M.R., Kristal, B.M., and Schanze, K.S. (2003) *Langmuir*, **19**, 6523–6533.
46 Qin, C., Cheng, Y., Wang, L., Jing, X., and Wang, F. (2008) *Macromolecules*, **41**, 7798–7804.
47 Ho, H.A., Boissinot, M., Bergeron, M.G., Corbeil, G., Doré, K., Boudreau, D., and Leclerc, M. (2002) *Angew. Chem.*, **114**, 1618–1621.
48 Zhai, L. and McCullough, R.D. (2002) *Adv. Mater.*, **14**, 901–905.
49 Gutacker, A., Adamczyk, S., Helfer, A., Garner, L.E., Evans, R.C., Fonseca, S.M., Knaapila, M., Bazan, G.C., Burrows, H.D., and Scherf, U. (2010) *J. Mater. Chem.*, **20**, 1423–1430.
50 Balanda, P.B., Ramey, M.B., and Reynolds, J.R. (1999) *Macromolecules*, **32**, 3970–3978.
51 Ramey, M.B., Ann Hiller, J., Rubner, M.F., Tan, C., Schanze, K.S., and Reynolds, J.R. (2005) *Macromolecules*, **38**, 234–243.
52 Stork, M., Gaylord, B.S., Heeger, A.J., and Bazan, G.C. (2002) *Adv. Mater.*, **14**, 361–366.
53 Yang, R., Wu, H., Cao, Y., and Bazan, G.C. (2006) *J. Am. Chem. Soc.*, **128**, 14422–14423.
54 Zhang, Z., Fan, Q., Sun, P., Liu, L., Lu, X., Li, B., Quan, Y., and Huang, W. (2010) *Macromol. Rapid Commun.*, **31**, 2160–2165.
55 Pu, K.Y., Cai, L.P., and Liu, B. (2009) *Macromolecules*, **42**, 5933–5940.
56 Yuan, H.X., Liu, Z., Liu, L.B., Lv, F.T., Wang, Y.L., and Wang, S. (2014) *Adv. Mater.*, **26**, 4333–4338.
57 He, F., Ren, X., Shen, X., and Xu, Q.-H. (2011) *Macromolecules*, **44**, 5373–5380.
58 Zhang, Y., Yang, Y., Wang, C.C., Sun, B., Wang, Y., Wang, X.Y., and Shen, Q.D. (2008) *J. Appl. Polym. Sci.*, **110**, 3225–3233.

59 Pu, K.-Y., Pan, S.Y.-H., and Liu, B. (2008) *J. Phys. Chem. B*, **112**, 9295–9300.
60 Pu, K.-Y., Li, K., Shi, J., and Liu, B. (2009) *Chem. Mater.*, **21**, 3816–3822.
61 Fukuhara, G. and Inoue, Y. (2011) *J. Am. Chem. Soc.*, **133**, 768–770.
62 Henson, Z.B., Zhang, Y., Nguyen, T.-Q., Seo, J.H., and Bazan, G.C. (2013) *J.Am. Chem. Soc.*, **135**, 4163–4166.
63 Rubio-Magnieto, J., Thomas, A., Richeter, S., Mehdi, A., Dubois, P., Lazzaroni, R., Clément, S., and Surin, M. (2013) *Chem. Commun.*, **49**, 5483–5485.
64 Twomey, M., Mendez, E., Manian, R.K., Lee, S., and Moon, J.H. (2016) *Chem. Commun. (Camb.)*, **52**, 4910–4913.
65 Aslund, A., Herland, A., Hammarstrom, P., Nilsson, K.P.R., Jonsson, B.H., Inganas, O., and Konradsson, P. (2007) *Bioconjug. Chem.*, **18**, 1860–1868.
66 Costa, T., de Azevedo, D., Stewart, B., Knaapila, M., Valente, A.J.M., Kraft, M., Scherf, U., and Burrows, H.D. (2015) *Polym. Chem.*, **6**, 8036–8046.
67 Fang, J., Wallikewitz, B.H., Gao, F., Tu, G., Muller, C., Pace, G., Friend, R.H., and Huck, W.T. (2011) *J. Am. Chem. Soc.*, **133**, 683–685.
68 Li, J., Tian, C., Yuan, Y., Yang, Z., Yin, C., Jiang, R., Song, W., Li, X., Lu, X., Zhang, L., Fan, Q., and Huang, W. (2015) *Macromolecules*, **48**, 1017–1025.
69 Phillips, R.L., Kim, I.-B., Carson, B.E., Tidbeck, B.r., Bai, Y., Lowary, T.L., Tolbert, L.M., and Bunz, U.H. (2008) *Macromolecules*, **41**, 7316–7320.
70 Zhou, L., Geng, J., Wang, G., Liu, J., and Liu, B. (2013) *Polym. Chem.*, **4**, 5243–5251.
71 Pu, K.Y., Li, K., and Liu, B. (2010) *Adv. Funct. Mater.*, **20**, 2770–2777.
72 Liu, J., Ding, D., Geng, J., and Liu, B. (2012) *Polym. Chem.*, **3**, 1567–1575.
73 Liu, J., Geng, J., and Liu, B. (2013) *Chem. Commun.*, **49**, 1491–1493.
74 Pecher, J. and Mecking, S. (2010) *Chem. Rev.*, **110**, 6260–6279.
75 Kurokawa, N., Yoshikawa, H., Hirota, N., Hyodo, K., and Masuhara, H. (2004) *Chemphyschem*, **5**, 1609–1615.
76 Szymanski, C., Wu, C.F., Hooper, J., Salazar, M.A., Perdomo, A., Dukes, A., and McNeill, J. (2005) *J. Phys. Chem. B*, **109**, 8543–8546.
77 Li, K., Pan, J., Feng, S.S., Wu, A.W., Pu, K.Y., Liu, Y., and Liu, B. (2009) *Adv. Funct. Mater.*, **19**, 3535–3542.
78 Hashim, Z., Howes, P., and Green, M. (2011) *J. Mater. Chem.*, **21**, 1797–1803.
79 Wu, C., Schneider, T., Zeigler, M., Yu, J., Schiro, P.G., Burnham, D.R., McNeill, J.D., and Chiu, D.T. (2010) *J. Am. Chem. Soc.*, **132**, 15410–15417.
80 Wu, C., Jin, Y., Schneider, T., Burnham, D.R., Smith, P.B., and Chiu, D.T. (2010) *Angew. Chem. Int. Ed.*, **49**, 9436–9440.
81 Yu, J., Wu, C., Zhang, X., Ye, F., Gallina, M.E., Rong, Y., Wu, I.C., Sun, W., Chan, Y.H., and Chiu, D.T. (2012) *Adv. Mater.*, **24**, 3498–3504.
82 Kandel, P.K., Fernando, L.P., Ackroyd, P.C., and Christensen, K.A. (2011) *Nanoscale*, **3**, 1037–1045.
83 Liu, J., Feng, G., Ding, D., and Liu, B. (2013) *Polym. Chem.*, **4**, 4326–4334.
84 Ding, D., Li, K., Qin, W., Zhan, R., Hu, Y., Liu, J., Tang, B.Z., and Liu, B. (2013) *Adv. Healthc. Mater.*, **2**, 500–507.
85 Almeida, C.S., Herrmann, I.K., Howes, P.D., and Stevens, M.M. (2015) *Chem. Mater.*, **27**, 6879–6889.
86 Geng, J., Liu, J., Liang, J., Shi, H., and Liu, B. (2013) *Nanoscale*, **5**, 8593–8601.