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Enantiomeric Differentiation by Synthetic Helical Polymers

Eiji Yashima, Hiroki Iida, and Yoshio Okamoto

Abstract Recent advances in the synthesis of helical polymers and their applications as chiral materials, in particular chiral stationary phases (CSPs), for high-performance liquid chromatography (HPLC) are reviewed with an emphasis on the key role of the helical conformations with one-handedness for the prominent chiral recognition of enantiomers. The historical background of artificial optically active helical polymers is also briefly described.

Keywords Chiral recognition · Chiral stationary phase · Enantioseparation · Helical macromolecules · Helix

Contents

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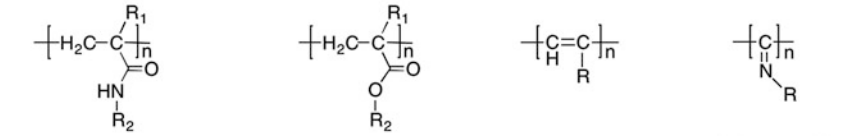
1 Introduction

Who prepared helical polymers for the first time? It may be a difficult question, but it may not be emphasized enough that in 1955 Natta discovered the isotactic polypropylene that formed a helical structure in the crystalline state when polymerized using the Ziegler–Natta catalyst [1]. Although the helical conformation of the polypropylene consists of a mixture of right- and left-handed helices and changes into a random conformation once dissolved in solvents due to the insufficient steric requirement of the side groups, this finding was a significant milestone, through which remarkable progress has been achieved in the field of synthetic helical polymers during the past three decades. At that time, however, there was almost no prospect for applications of these helical polymers.

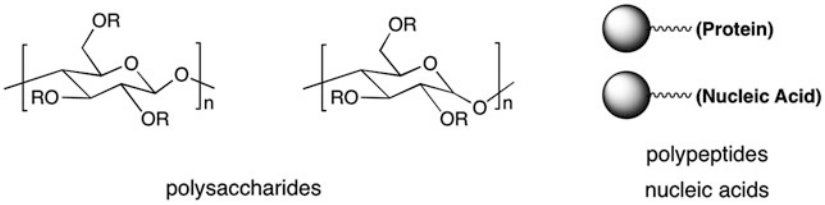
The first innovative research was reported in 1979 [2]; Okamoto et al. developed a helical polymethacrylate synthesized by helix-sense-selective polymerization using an optically active initiator that showed an excellent chiral recognition ability for aromatic racemic compounds when used as a chiral stationary phase (CSP) for high-performance liquid chromatography (HPLC). Bulky ester groups introduced at the side groups force the polymer to maintain its helical conformation in solution. Since then, a number of optically active helical polymers have been prepared and applied as CSPs for HPLC. Importantly, prior to this discovery, Blaschke et al. prepared optically active polyacrylamides and polymethacrylamides by the radical polymerization of the corresponding monomers with chiral pendants, which resolved many racemates, particularly chiral drugs, in liquid chromatography (LC) to reveal the differences in the pharmacological and physiological behaviors of both drug enantiomers [3]. These results will be described in more detail below.

Nowadays it is recognized that thorough investigations of such pharmacological and physiological actions of both enantiomers of chiral drugs are essential before being commercialized. The separation of enantiomers using CSPs by HPLC is of key importance for determining their enantiomeric excess (ee) and also for obtaining pure enantiomers on analytical and industrial scales [4–14]. At present, many CSPs for HPLC that are commercially available involve small chiral molecule-based CSPs [15–20] and chiral polymer-based CSPs derived from polysaccharides [21–28], proteins [29–31], and synthetic helical polymers [3, 32, 33]. Among them, the helical polymer-based CSPs, particularly CSPs composed of synthetic helical polymers with a controlled helical sense, and cellulose and amylose derivatives [21–28], are particularly interesting because their chiral recognition abilities are dependent on their highly-ordered helical structures that are responsible for their powerful resolving abilities. Therefore, in this chapter we focus on the recent advances in the synthesis of helical polymers and their applications, mainly as CSPs for HPLC.

Synthetic Helical Polymers



Natural Helical Polymers and Their Derivatives



2 Chiral Recognition by Synthetic Helical Polymers

2.1 Poly(meth)acrylamides

Before the 1980s, Blaschke and coworkers systematically synthesized a series of optically active polyacrylamides and polymethacrylamides (**1**–**4**) by radical polymerization of the corresponding monomers, and investigated their chiral recognition abilities as CSPs for LC [3]. In those days, the chirality of drugs was often neglected, and many chiral drugs were put on the market as racemates. Blaschke et al. successfully resolved many chiral pharmaceutical compounds using optically active polyacrylamide gels on a preparative scale and investigated the differences in the pharmacological behaviors between the enantiomers. For example, (±)-thalidomide (**6**), which had been used as a hypnotic and sedative drug and caused a terrible tragedy due to its potent teratogenicity, was for the first time completely resolved into its enantiomers using their CSPs including **2** (Fig. 1). They reported that the teratogenic action was caused only by the *S* enantiomer, whereas the *R* enantiomer did not show any teratogenic behavior, even at high doses [34]. Although this result stirred controversy arising from the fact that each enantiomer readily racemizes at physiological pH or after injection into rabbits [35, 36], this study is of key importance even now, since legislative authorities have now recognized the importance of evaluating the different pharmacological behaviors of both enantiomers of a drug before marketing. Up to now, diverse optically active poly(meth)acrylamides involving **1**–**5** have been prepared for the development of more efficient CSPs with wide applicability. For example, **3** could resolve various racemates, such as **7**–**9**, and has been

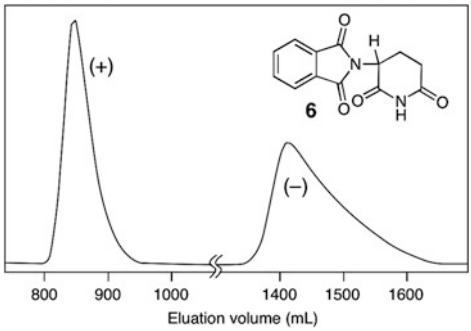
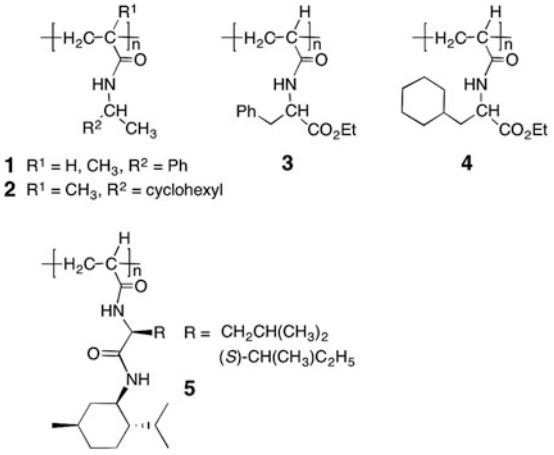
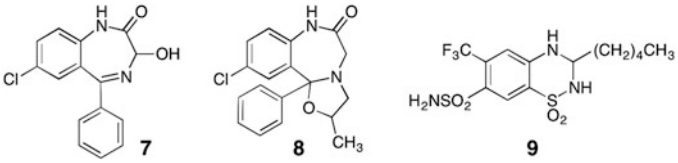


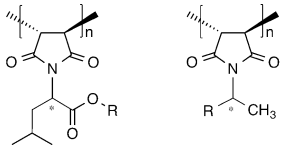
Fig. 1 Chromatographic resolution of **6** on **2**. Eluent: benzene/dioxane (8/2, v/v). (Reproduced with permission from [3]. Copyright 1980 Wiley-VCH)

commercialized under the trade name of Chiraspher (Merck) [37]. The structures of polymethacrylamides used as chiral packing materials remain unknown because most of the polymers were prepared by radical polymerization, thus mostly producing atactic polymers. However, Blaschke et al. found that the chiral recognition abilities of polymethacrylamides depend on the synthetic route. The polymers prepared by radical polymerization of the corresponding optically active methacrylamides showed much better chiral recognition than those prepared from poly(acryloyl chloride) followed by the reaction with chiral amines [3]. These results suggest that chiral recognition sites in the CSPs derived from the polymethacrylamides may be kinetically produced, implying a possible higher order structure, such as a helical conformation in part induced by the optically active pendant groups that may be formed during the polymerization process. Interestingly, it was also reported that the tacticity of polymethacrylamides is an important factor for their chiral recognition abilities [38].





Poly(*N*-substituted maleimide)s bearing various optically active pendant groups, such as **10** and **11**, prepared by the anionic polymerization of the corresponding monomers with chiral catalysts or initiators often showed a relatively high optical rotation, probably due to a helical conformation with a preferential helical sense [39–42]. However, there is another possibility to explain the optical activity of the poly(*N*-substituted maleimide)s. In contrast to vinyl polymers prepared from 1-substituted and 1,1-disubstituted vinyl monomers, including poly(meth)acrylamides and polymethacrylates, poly(maleimide)s can be optically active due to truly chiral stereogenic centers in the main-chain when the polymerization produces one of two enantiomeric *trans* structures (**10** and **11**). The vinyl polymers, even if they possess highly stereoregular isotactic or syndiotactic structures, whose stereogenic centers in the main-chain are pseudoasymmetric because the whole polymer chain has a mirror plane, are optically inactive. The poly(*N*-substituted maleimide)s involving **11** were able to resolve enantiomers, such as 1,1′-bi-2-naphthol, when used as an HPLC packing material that was also commercialized.



2.2 Polymethacrylates

Polymethacrylates with a one-handed helical structure belong to an important class of polymer-based CSPs. Okamoto et al. prepared the first helical vinyl polymer by helix-sense-selective polymerization of an achiral (prochiral) bulky methacrylate, i.e., triphenylmethyl methacrylate (TrMA), using chiral anionic initiators, such as *N,N*′-diphenylethylenediamine monolithium amide (DPEDA-Li) complexed with chiral ligands, such as (+)-2,3-dimethoxy-1,4-bis(dimethylamino) butane (DDB), giving a single-handed, fully isotactic helical polymer with a high optical rotation (PTrMA) (Fig. 2a) [2, 43]. The chiral ligand coordinating to the living propagating polymer end controls the main-chain configuration as well as the helical sense of the entire polymer chain, resulting from the bulky triphenylmethyl groups that

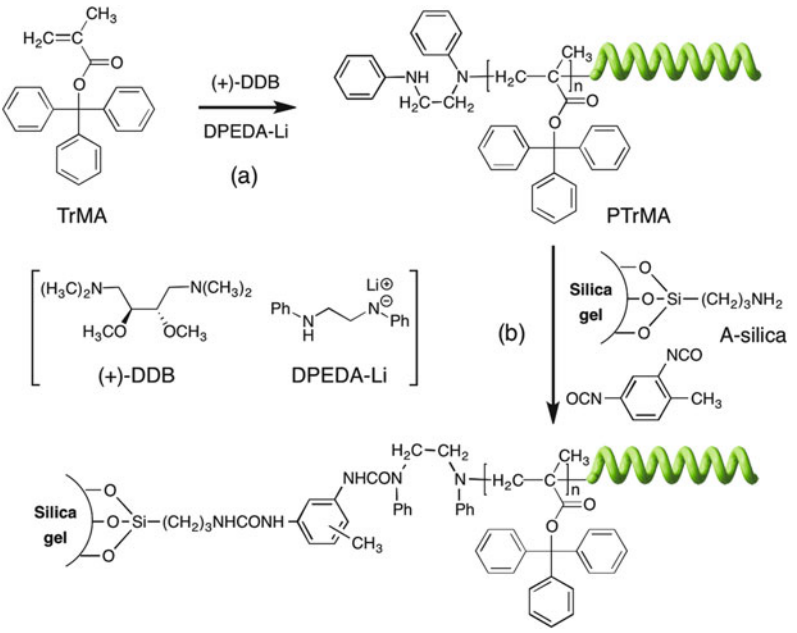


Fig. 2 (**a**) Helix-sense-selective polymerization of TrMA using chiral anionic initiators. (**b**) Preparation of PTrMA-immobilized CSP using A-silica and toluen-2,4-diyldiisocyanate

prevent the polymer from unfolding or racemization of the helical conformation kinetically formed during the polymerization process. The optically active helical PTrMA showed an excellent chiral recognition for a variety of racemic compounds when coated on a macroporous silica gel and used as a CSP for HPLC [44], and the PTrMA-based CSP has been commercialized (CHIRALPAK OT(+)). In particular, the PTrMA-based CSP is useful for the enantiomeric differentiation of stereochemically interesting aromatic racemates [45] including **12**–**18** [46–52], which are difficult to resolve by other CSPs or conventional methods. Polar eluents, such as methanol and a methanol–water mixture, are often used for better resolution, indicating that hydrophobic interactions between nonpolar groups of a solute and the side groups (triphenylmethyl groups) of PTrMA play an important role. It has been proposed that the triphenylmethyl pendant groups take a propeller-like chiral structure along the helically twisted polymer main-chain, which contributes to its quite high chiral recognition [53].

Figure 3 shows a chromatogram of the resolution of racemic 1,1′-bi-2-naphthol (**19**) on PTrMA, where the (+)-enantiomer elutes first, followed by the (−)-enantiomer at retention times of t_1 and t_2, respectively, showing complete baseline separation [44]. The results of the chromatographic differentiation of enantiomers can be evaluated using the following three parameters: retention factor k_1' [$= (t_1 - t_0)/t_0$], separation factor α [$= (t_2 - t_0)/(t_1 - t_0)$], and resolution factor R_S [$= 2(t_2 - t_1)/(w_1 + w_2)$], where t_0 is the dead time (retention time of a non-retained compound),

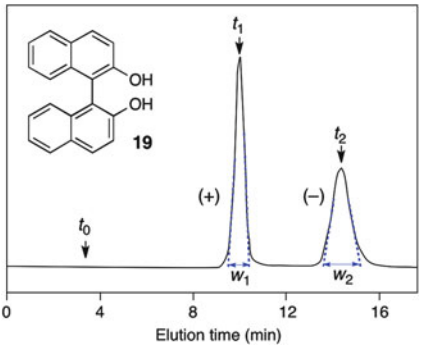
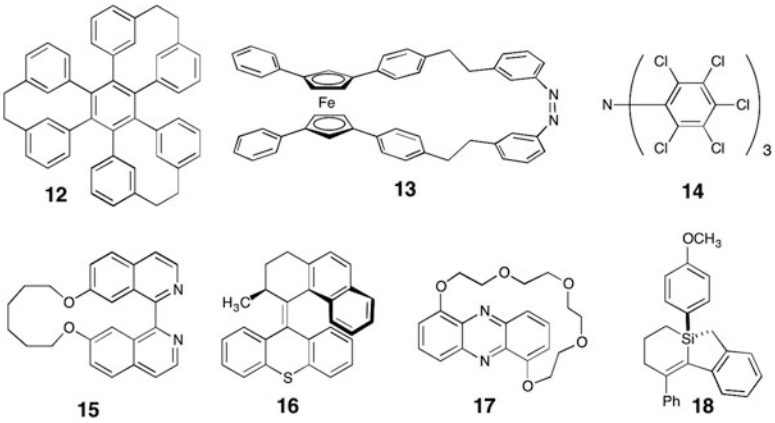
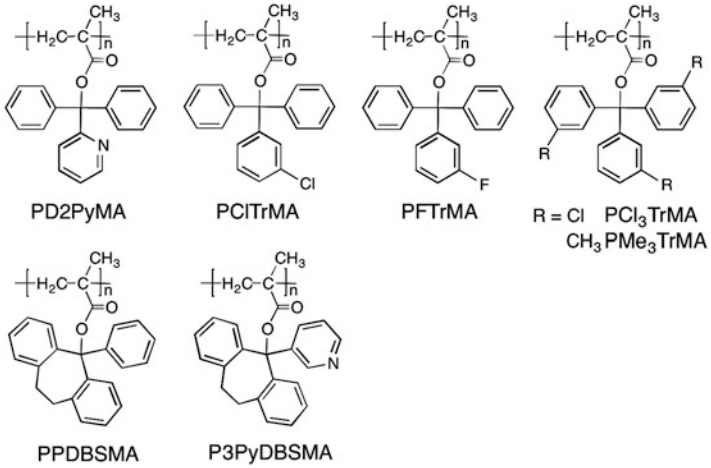


Fig. 3 Chromatographic resolution of **19** on PTrMA. Eluent: methanol. (Reproduced with permission from [44]. Copyright 1981 American Chemical Society)

and w_1 and w_2 are the bandwidths of the peaks. For a chromatographic enantioseparation, α is directly related to the chiral recognition ability of a CSP, and R_S reflects both the chiral recognition ability of a CSP and the column efficiency (theoretical plate number).



PTrMA is among the popular chiral polymer-based CSPs, but has a drawback that the triphenylmethyl ester groups are slowly solvolyzed in methanol when used as an eluent during HPLC separation, and the polymer gradually loses its helical conformation, resulting in a loss of its chiral recognition ability. Therefore, a one-handed helical PD2PyMA bearing diphenylpyridylmethyl groups as the pendants was prepared by the helix-sense-selective anionic polymerization to improve its durability, since the corresponding monomer is more robust against solvolysis than TrMA, providing a more practically useful CSP. In fact, the methanolysis of PD2PyMA-coated silica gel in methanol at 60°C was 16-fold slower than that of the PTrMA-coated one [54]. The PD2PyMA-based CSP exhibited a slightly lower resolution ability than that of the PTrMA-based CSP under the same chromatographic conditions, but several 1,1′-bi-2-naphthyl derivatives were better resolved on PD2PyMA than PTrMA [54].



The related meta-halogen- and methyl-substituted optically active PTrMA-derivatives were also prepared and their chiral recognition abilities as CSPs were investigated [55, 56]. The chiral recognition abilities of PClTrMA and PFTrMA were slightly low in comparison to that of PTrMA, and PCl_3TrMA exhibited almost no resolving ability, although introduction of the electron-withdrawing halogen groups on the triphenylmethyl pendants makes the PTrMA derivatives robust against solvolysis of the ester groups. PMe_3TrMA also possessed a high chiral recognition ability, resolving some racemates more efficiently than PTrMA, but the introduction of such an electron-donating methyl group caused a decrease in durability. PPDBSMA and P3PyDBSMA bearing a dibenzosuberyl group as the pendant also had a higher durability against solvolysis compared to PTrMA, but their chiral recognition abilities were rather low [57].

Optically active PTrMA chemically bonded to silica gel was also prepared by the reaction of PTrMA bearing one DPEDA-derived $PhNHCH_2CH_2$- terminal group with 3-aminopropyl-silanized silica gel (A-silica) that were cross-linked using toluen-2,4-diyldiisocyanate (Fig. 2b). The resulting PTrMA-immobilized CSP exhibited a chiral recognition ability similar to that of the corresponding coated-type CSP when methanol was used as an eluent. Interestingly, the chemically bonded CSP partially resolved the racemic helical polymers composed of an equimolar mixture of right- and left-handed helices, such as PTrMA [58], PD2PyMA [59], and PPDBSMA [60], into the enantiomers with a single-handed helical conformation as shown in Fig. 4.

A unique approach to create a chiral cavity using helical polymers as a template has been reported based on a molecular imprinting method. Optically active single-handed helical P3PyDBSMA was used as a template and further cross-linked by radical copolymerization to produce a gel (Fig. 5) [61]. After removal of the template, the gel maintained its optical activity and preferentially adsorbed the P3PyDBSMA with the same helical sense used as the template. The gel also could recognize the chirality for some racemates, such as *trans*-stilbene oxide, and enantioselectively adsorbed one of the enantiomers, whereas the gel showed no

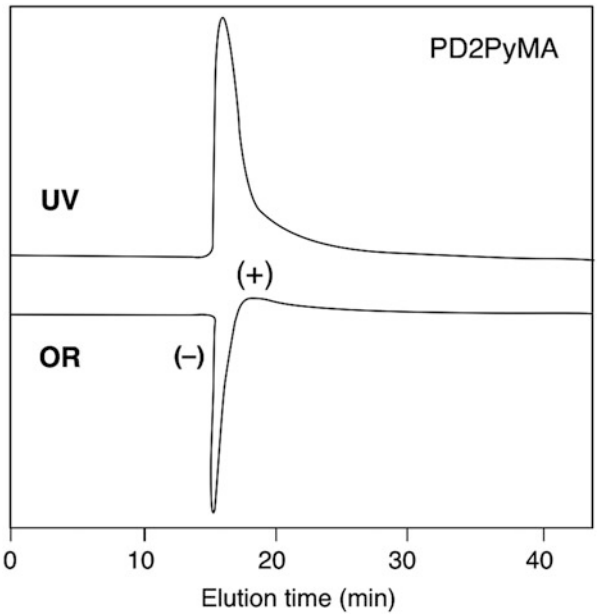


Fig. 4 Chromatographic resolution of racemic PD2PyMA on PTrMA. Eluent: $CHCl_3$. (Reproduced with permission from [59]. Copyright 1989 American Chemical Society)

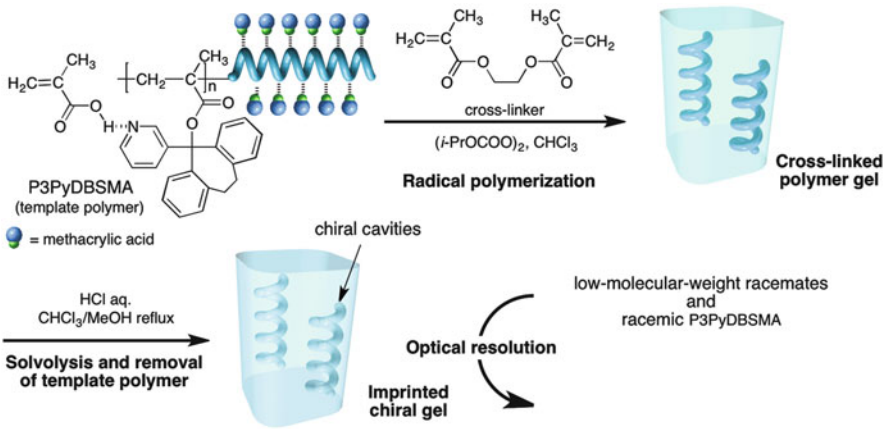
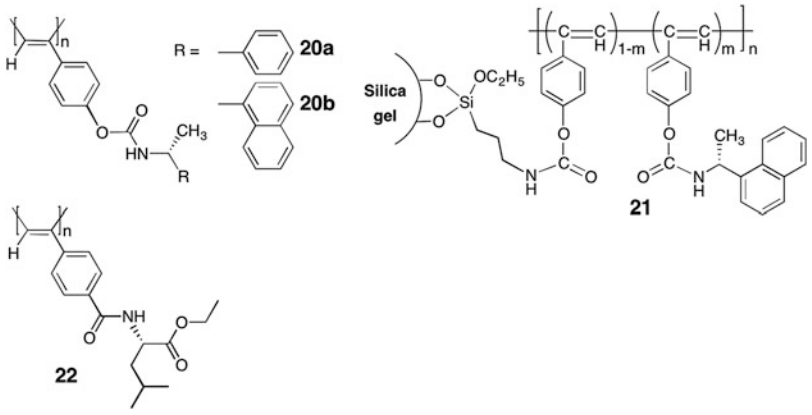


Fig. 5 Schematic illustrations of the chiral gel synthesis by a molecular imprinting method using a single-handed helical P3PyDBSMA as a template. After cross-linking of methacrylic acid using a cross-linker, P3PyDBSMA is removed to produce an imprinted chiral gel

chiral recognition toward *trans*-stilbene oxide before removal of the template, suggesting that the helical structure of the template is indeed "imprinted" [62, 63] in the gel and the observed resolution is most likely based on the chiral cavity imprinted in the gel.

2.3 Polyacetylenes

Since 1967, a large number of helical polyacetylenes with optical activities mediated by the enantiomerically pure pendants has been prepared mostly by the transition metal-catalyzed polymerization of optically active acetylenes. Among them, phenylacetylenes bearing enantiomerically pure pendant groups have the ability to be designed and synthesized, and readily polymerize using a rhodium catalyst, giving stereoregular (*cis-transoidal*) high molecular weight poly (phenylacetylene)s with an excess of a one-handed helical sense [64–68]. An optically active poly(phenylacetylene) bearing (*R*)-phenylethylcarbamoyl pendant groups **20a** was the first helical polyacetylene to have its chiral recognition ability evaluated as a CSP for HPLC after coating on a macroporous silica gel. The CSP efficiently resolved several racemates [69]. The precise control of the main-chain stereoregularity is essential for induction of a preferred-handed helical conformation and the resulting chiral recognition as well. This was revealed by the fact that a stereoirregular poly(phenylacetylene) with a chemical structure identical to **20a**, prepared by a different synthetic route, exhibited poor chiral recognition. A similar, but chemically bonded-type CSP **21** has also been prepared by the copolymerization of the corresponding chiral monomer with a rhodium catalyst in the presence of silica gel with phenylacetylene residues chemically bonded on a silica surface. The CSP completely resolved several racemates, including **23** (Fig. 6) [70]. Both the chemically and physically immobilized CSPs derived from **20b** also showed relatively high and similar chiral recognition ability to each other.



Cinchona alkaloids are naturally-occurring optically active compounds each consisting of two pseudoenantiomeric forms (diastereomers), such as cinchonidine (**Cd**)/cinchonine (**Cn**) and quinine (**Qn**)/quinidine (**Qd**). They have been extensively used not only as versatile chiral organocatalysts [71, 72] but also as CSPs after modification and being chemically bonded to silica gel. The cinchona alkaloid-derived CSPs showed powerful chiral recognition abilities for acidic racemates

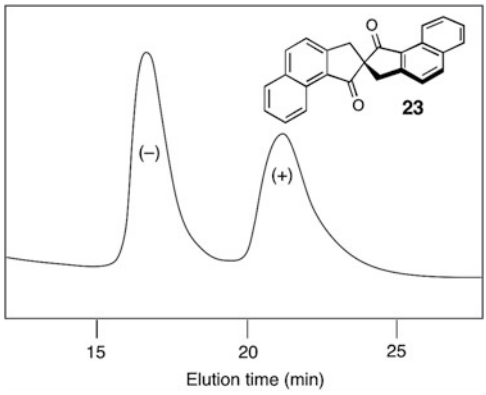


Fig. 6 Chromatographic resolution of **23** on **21**. Eluent: methanol. (Reproduced with permission from [70]. Copyright 1996 The Polymer Society of Korea)

under anion-exchange HPLC conditions [19], and some of them have been commercialized. A series of optically active helical poly(phenylacetylene)s bearing amino-functionalized cinchona alkaloid pendant groups connecting to the phenyl rings through an amide linkage were prepared with the expectation that the cinchona alkaloid pendants are capable of inducing a one-handed helical conformation of the polymers (Fig. 7a), so that the enantioselectivities would be further enhanced as compared to those of the cinchona alkaloids when used as chiral organocatalysts [73] and CSPs. The polymers were coated on a macroporous silica gel to obtain novel CSPs [74]. The circular dichroism (CD) spectral patterns between poly-**ACd** and poly-**ACn** (Fig. 7b) and also poly-**AQn** and poly-**AQd** were almost mirror images of each other due to their pseudoenantiomeric relationships, suggesting an opposite helical sense induced by the amide-linked cinchona alkaloid pendants. These helical polymers could resolve various racemic compounds into enantiomers including alcohols, diols, metal tris(acetylacetonato)s, and *N*-Boc-amino acids; in particular, poly-**ACd** and poly-**ACn** exhibited excellent chiral recognition abilities for the tested racemates (Fig. 8a). The reversed elution order was observed for racemates resolved on the pseudoenantiomeric poly-**ACd** and poly-**ACn** (Fig. 7c,d). In order to confirm the importance of the macromolecular helicity on the chiral recognition, *trans*-enriched poly-**ACn** (poly-**ACn′**) was prepared by grinding the *cis*-poly-**ACn**. Poly-**ACn′** showed a poor chiral recognition ability (Fig. 8b) because the polymer almost lost its helical conformation, resulting in the almost complete disappearance of the CD in the polymer backbone regions (Fig. 7b). These results clearly indicated that the chiral recognition ability was significantly influenced by the macromolecular helicity induced by the alkaloid pendants. Interestingly, these optically active helical polymers can be employed as an efficient polymeric organocatalyst for the asymmetric Henry reaction [73, 75]. Stereoregular poly(phenylacetylene)s bearing L-leucine ethyl ester side groups, such as **22**, also resolved some racemic compounds as CSPs for HPLC [76]. Interestingly, their resolving ability was significantly influenced by the solvents used for coating the polymer on the silica gel, because the helical conformations of poly(phenylacetylene)s are dynamic in nature, and the helical poly(phenylacetylene)s may take a different conformation, for example, an opposite helicity via inversion of helices, depending on the coating solvents.

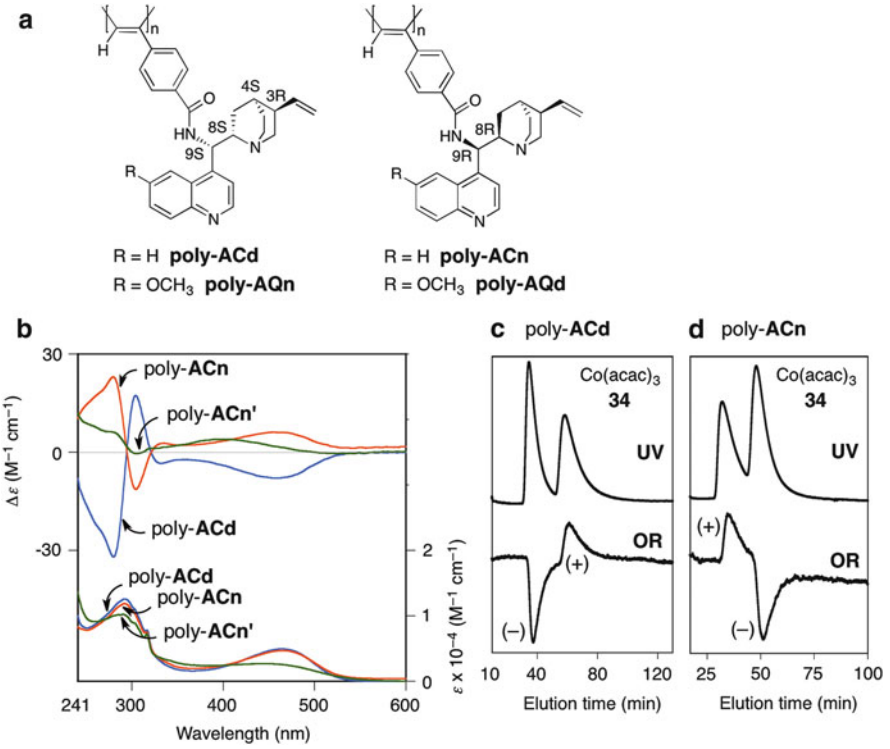
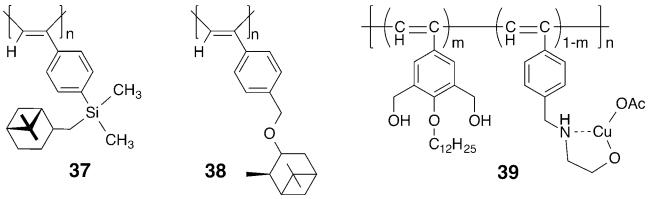


Fig. 7 (**a**) Structures of cinchona alkaloid-bound helical poly(phenylacetylene)s. (**b**) CD and absorption spectra of poly-**ACn**, poly-**ACd**, and poly-**ACn**′ measured in $CHCl_3$–CF_3CH_2OH (6:1, v/v) at 25 °C. (**c**,**d**) Chromatograms for the resolution of **34** on poly-**ACd** (**c**) and poly-**ACn** (**d**). Eluent: hexane-2-propanol (9:1, v/v). (Reproduced with permission from [74]. Copyright 2012 The Chemical Society of Japan)

Helical poly(phenylacetylene)s having chiral pinanyl groups (**37** and **38**) [77, 78] and hydrophilic *N*-(2-hydroxyethyl)aminomethyl groups, being capable of chelating to Cu^{2+} ion as the pendants (**39**) [79], have also been prepared and used as solid membranes for separating the enantiomers of amino acids through permeation. Helical polymer-based solid membranes have a significant potential and are attractive from the continuous and preparative standpoints for development of a practically useful new chiral separation method, but require further improvements in efficiency and enantioselectivity before practical use.



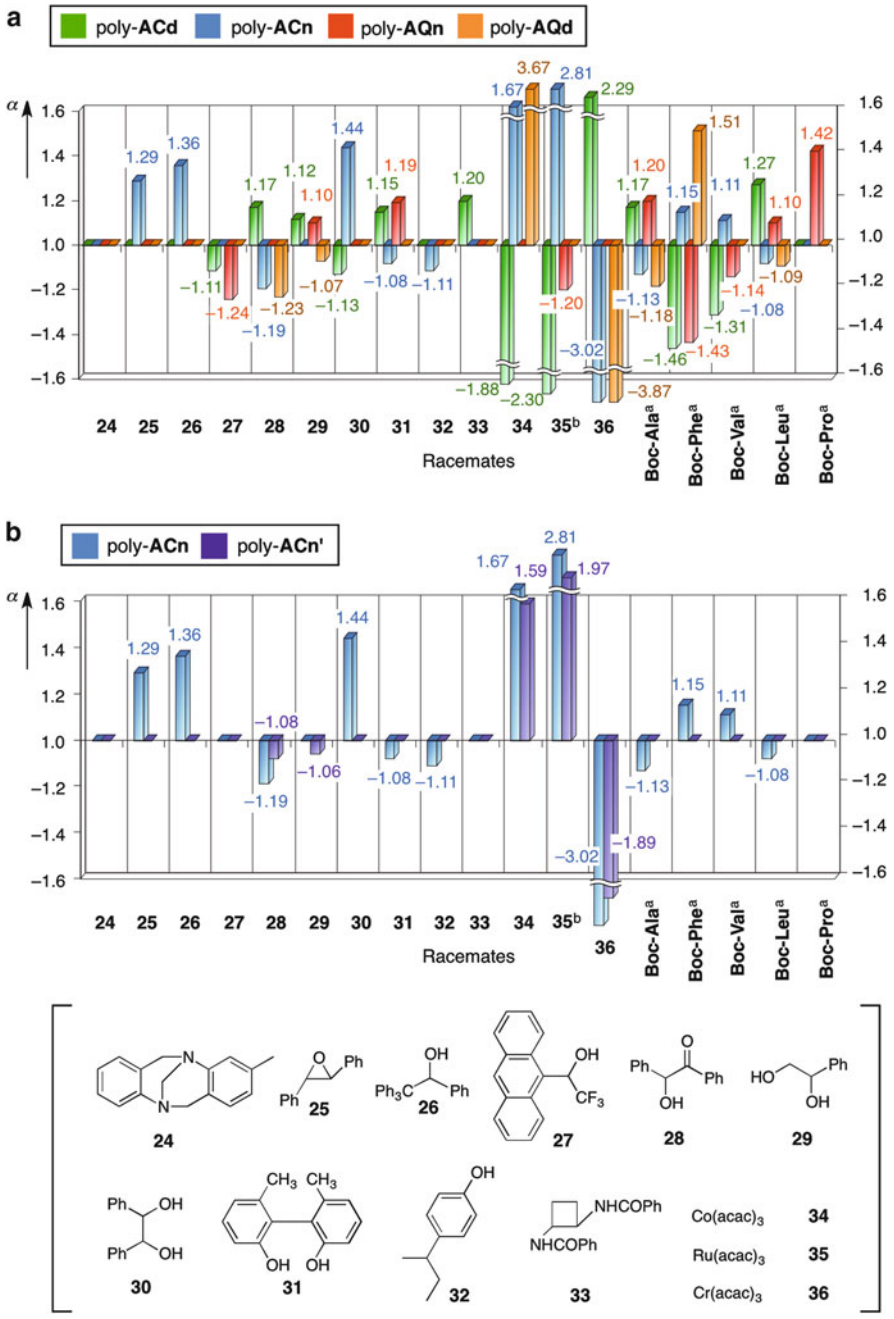
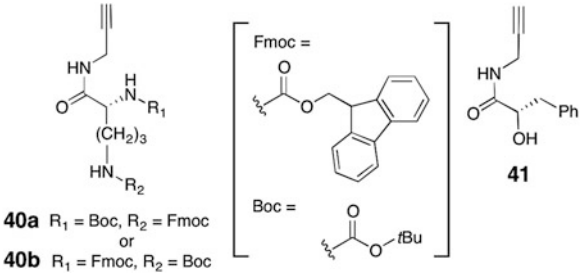


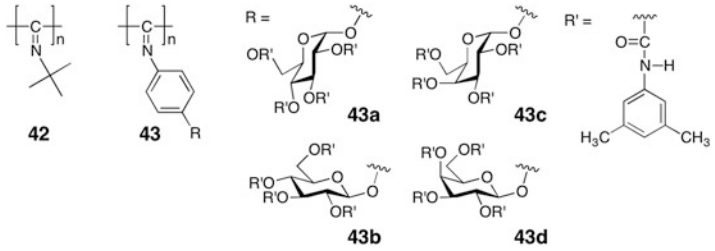
Fig. 8 Histograms of the separation factors (α) on poly-**ACd**, poly-**ACn**, poly-**AQn**, and poly-**AQd** (**a**) and poly-**ACn** and poly-**ACn′** (**b**). Eluent: hexane–2-propanol (9:1, v/v). The signs of the vertical axis (α value) represent the optical rotation of the first-eluted enantiomers. [a]Eluent: hexane–2-propanol (9:1, v/v) containing 2% acetic acid. [b]The signs of the vertical axis (α value) represent the CD detection (254 nm) of the first-eluted enantiomer

Optically active polyacetylene gels prepared by the copolymerization of L-lysine-derived *N*-propargylamides (**40a** and **40b**) with an achiral diacetylene or dipropargyl adipate as a cross-linker using a rhodium catalyst enantioselectively adsorbed *N*-benzyloxycarbonyl L-Ala derivatives and a chiral diol [80]. A similar gel derived from an optically active *N*-propargylamide (**41**) and a bifunctional achiral acetylene monomer also adsorbed one of the enantiomers of the racemic phenylethanol and benzoin [81].



2.4 Polyisocyanides

Polyisocyanides with a bulky side group possess a stable helical conformation in solution as revealed by the direct resolution of poly(*tert*-butyl isocyanide) (**42**) into enantiomeric right- and left-handed helices by column chromatography using an optically active poly((−)-*sec*-butyl isocyanide) as a CSP [82]. This study clearly indicated the potential of helical polyisocyanides as CSPs. In fact, some racemates, such as $Co(acac)_3$ (**34**), were resolved in part when optically active **42** prepared by the helix-sense-selective polymerization was used as a CSP for HPLC [83]. As mentioned in Sect. 3.1, the 3,5-dimethylphenylcarbamates of cellulose and amylose are the most popular CSPs among the wide variety of commercially available CSPs. Accordingly, 3,5-dimethylphenylcarbamate-modified glucose- and galactose-carrying helical poly(phenyl isocyanide)s (**43**) have been prepared, and their chiral recognition abilities as CSPs for HPLC were evaluated [84]. The helical polyisocyanides completely or partially separated different types of racemates with functional groups depending on the stereostructure of the pendant sugar residues. Importantly, a non-helical vinyl polymer bearing the identical sugar units showed a lower chiral recognition, thus demonstrating the important role of the helical structures of **43**. Among the poly(phenyl isocyanide)-based CSPs, the galactose-carrying poly(phenyl isocyanide)s (**43c** and **43d**) showed a better resolving ability for broader racemates than those having the glucose-derived pendant groups (**43a** and **43b**).



Helical polymers with a controlled helical sense can be readily synthesized by the polymerization of specific optically active monomers, such as substituted isocyanides and acetylenes as described in the previous sections. Chiral residues introduced in the monomers determine their helical senses. Recently, the unprecedented helix-sense-controlled living polymerization has been found for the polymerization of one enantiomer of phenyl isocyanide (L-**44**) bearing an L-alanine pendant with a long *n*-decyl chain through an amide linkage using the μ-ethynediyl Pt-Pd catalyst (**45**), which produced both diastereomeric right (*P*)- and left (*M*)-handed helical polyisocyanides with different molecular weights and narrow molecular weight distributions (*P*- and *M*-poly-**44**s, respectively) [85, 86]. Each single-handed, rodlike helical polyisocyanide stabilized by intramolecular hydrogen-bonding networks through the neighboring amide NH groups [87] was easily separated by solvent fractionation using acetone (Fig. 9a) [88]. Quite interestingly, the helical structures of the fractionated *M*- and *P*-poly-**44**s including helical pitch, helical sense (right- and left-handed helices), and excess handedness were for the first time visualized and determined by high resolution atomic force microscopy (AFM) (Fig. 9b,c) [88] when the polymers were deposited on a graphite substrate under organic solvent vapors. The fractionated single-handed helical polyisocyanides (*P*- and *M*-poly-**44**s) maintained their living features and can be used as an initiator for the further block copolymerizations of isocyanides [89]. This advantage was utilized to immobilize chemically these right- and left-handed helical polyisocyanides on silica gel and their chiral recognition abilities were investigated as CSPs for HPLC with anticipation that the *P*- and *M*-poly-**44**s having the same L-alanine residues as the pendants, but with the opposite polymer backbone helical conformation, could exhibit different chiral recognition abilities toward enantiomers, depending on their helical sense [90]. The CSP derived from the left-handed helical polyisocyanide (Si-*M*-BP(−)) successfully resolved racemates including a cyclic ether and dibenzamides, while the right-handed helical polyisocyanide-based CSP (Si-*P*-BP(+)) exhibited a rather complementary chiral recognition ability and specifically separated racemic metal acetylacetonate complexes, which could not be separated at all on the former CSP (Fig. 9d). Moreover, the reversed elution order was observed for some racemates resolved on the right- and left-handed helical polymer-based CSPs (Fig. 9e), thus indicating that the enantioselectivity and elution order of the enantiomers are significantly influenced by the helical sense of the polyisocyanides.

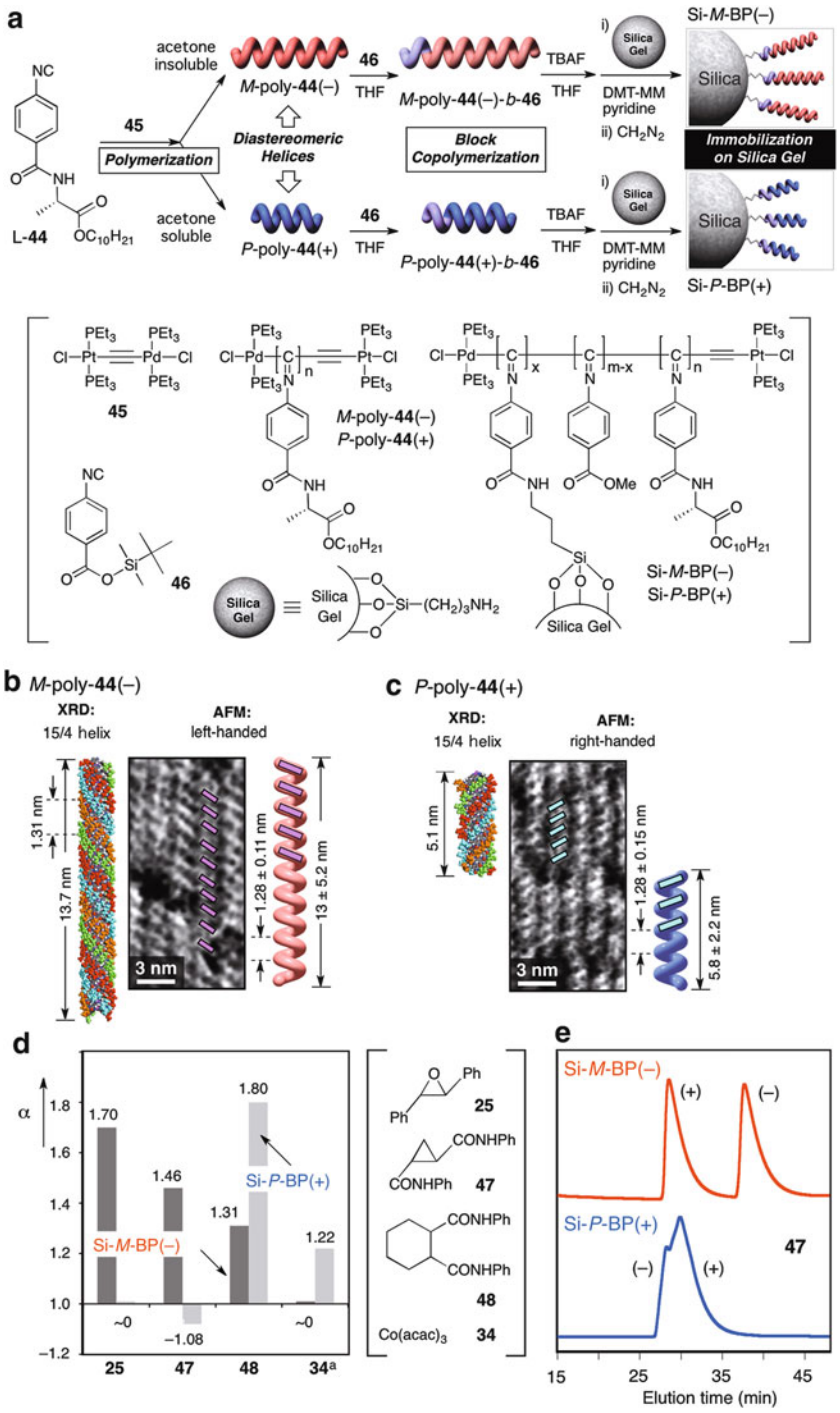


Fig. 9 (**a**) Synthesis of *M*-poly-**44**(−)-*b*-**46** and *P*-poly-**44**(+)-*b*-**46** and immobilization on silica gel. (**b**,**c**) AFM images of *M*-poly-**44**(−) (**b**) and *P*-poly-**44**(+) (**c**) on a highly oriented pyrolytic

When the left-handed helical polyisocyanide (*M*-poly-**44**(−)) was copolymerized with a bifunctional cross-linker (**49**), a unique optically active star polymer (*M*-poly-**44**(−)-*b*-**49**) was formed (Fig. 10a) and its star-shaped unique "spiny" architecture and its structure with individual helical arms including the number and length of the arms and its handedness have been directly visualized by high-resolution AFM (Fig. 10b) [91]. The star polymer (*M*-poly-**44**(−)-*b*-**49**) could discriminate the chirality of some racemates (**25**, **28**, and **50**) and a racemic helical polyisocyanide bearing no chiral pendants (poly-**51**), and enantioselectively adsorbed one of the enantiomers; the separation factors (α) calculated based on the enantioselective adsorption experiments were 1.81, 1.20, 1.45, and 1.14 respectively (Fig. 10c). Interestingly, the arm polyisocyanide (*M*-poly-**44**(−)) exhibited a poor chiral recognition ($\alpha = 1.03$) for poly-**51**. A confined chiral nano-space created in the star polymer through self-assembly of the helical arms may play a role in this helix-sense-selective adsorption of a large macromolecule.

Based on a facile method for constructing a helical polymer with a controlled helical sense via noncovalent bonding interactions, that is the "helicity induction and memory strategy" [66, 67, 92], discovered for the first time in poly(phenylacetylene)s [93] in 1999 and later in poly(phenyl isocyanide)s in 2004 [94–96], a series of optically active helical poly(phenyl isocyanide)s bearing achiral benzanilide pendant groups (poly(**53**–*co*-**54**-**Me**)s and poly-**55**) was prepared (Fig. 11a) [97]. The polymers possessing an optical activity solely due to the macromolecular helicity memory were chemically bonded to or coated on A-silica to prepare chiral packing materials for HPLC [97]. The CSPs resolved a variety of racemates; in particular, a right-handed helical poly(phenyl isocyanide) carrying achiral anilide pendants (*P*-poly-**55**(+)) showed an excellent resolving ability and completely or partially separated nine racemates into enantiomers, including a cyclic ether, amine, ketones, and metal acetylacetonate complexes, among the 12 racemates tested (Fig. 11b). The elution orders of the enantiomers were fully consistent with the helical senses of the polyisocyanides (*P* or *M*) as anticipated, because the optical activities of the polymers bearing achiral pendant groups result solely from the preferred-handed helical conformation with the macromolecular helicity memory.

Fig. 9 (continued) graphite (HOPG) substrate. Scale = 10 nm × 20 nm. Schematic representations of the left-handed helical *M*-poly-**44**(−) and right-handed helical *P*-poly-**44**(+) with periodic oblique stripes (*pink* and *blue lines*, respectively), which denote a one-handed helical array of the pendants and optimized 15/4 helical structures of *M*-poly-**44**(−) and *P*-poly-**44**(+) on the basis of X-ray structural analysis results, are also shown. (Reproduced with permission from [88]. Copyright 2008 American Chemical Society.) (**d**) Histograms of the separation factors (α) on Si-*M*-BP(−) and Si-*P*-BP(+). The signs of the vertical axis (α value) represent the optical rotation of the first-eluted enantiomers. Eluent: hexane–2-propanol (98:2, v/v). [a]Eluent: hexane–THF (98:2, v/v). The signs of the vertical axis (α value) represent the CD detection (254 nm) of the first-eluted enantiomer. (**e**) Chromatograms for the resolution of **47** on Si-*M*-BP(−) (*red lines*) and Si-*P*-BP(+) (*blue lines*). Eluent: hexane–2-propanol (98:2, v/v). (Reproduced with permission from [90]. Copyright 2011 The Royal Society of Chemistry)

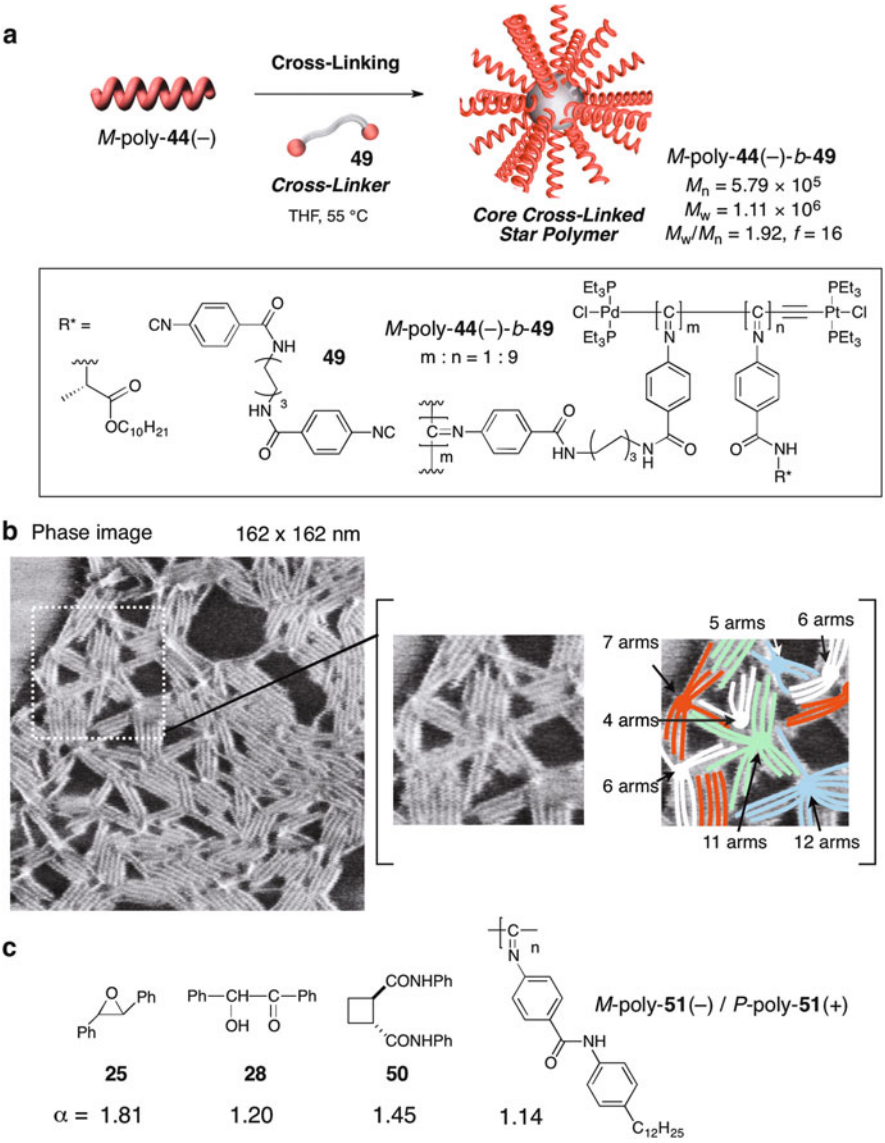


Fig. 10 (**a**) Synthesis of a star-shaped *M*-poly-**44**(−)-*b*-**49** with a cross-linker **49**. (**b**) AFM image (scale 162 nm × 162 nm) of 2D self-assembled *M*-poly-**44**(−)-*b*-**49** on HOPG. (**b**) Zoomed AFM image corresponding to the area indicated by a *white square*, and schematic representations of possible helix-bundle arrangements are also shown (*right*). Each molecule is indicated by different colors. (Reproduced with permission from [91]. Copyright 2011 American Chemical Society). (**c**) Results of enantioselective adsorption of racemic compounds (**25**, **28**, and **50**) and racemic helical polymer (poly-**51**) on *M*-poly-**44**(−)-*b*-**49**

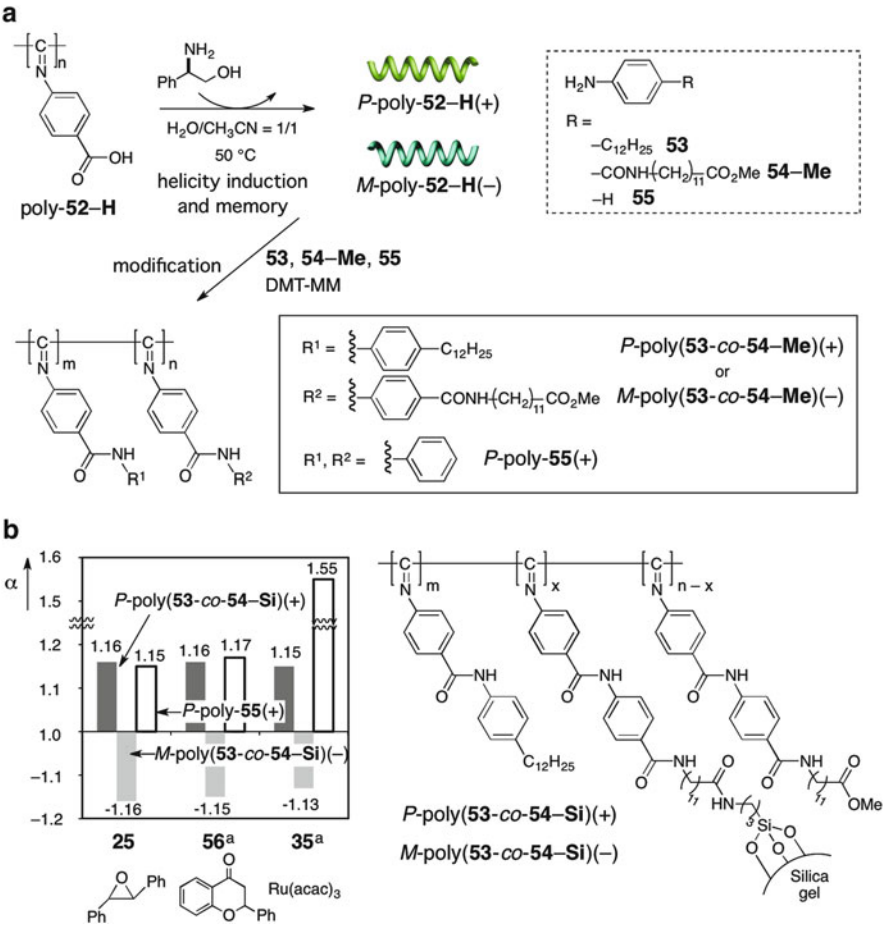


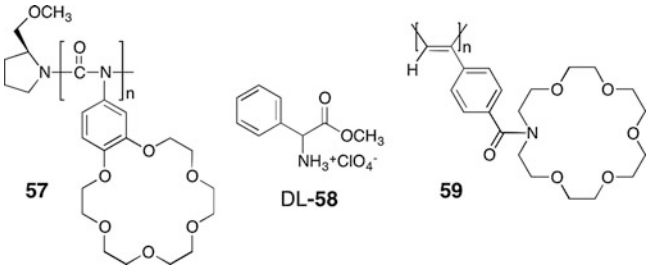
Fig. 11 (**a**) Synthesis of optically active helical poly(phenyl isocyanide)s bearing achiral benzanilide pendant groups (poly(**53**–*co*-**54**-**Me**)s and poly-**55**). (**b**) Histograms of the separation factors (α) on *P*-poly(**53**–*co*-**54**-**Si**)(+), *M*-poly(**53**–*co*-**54**-**Si**)(−), and *P*-poly-**55**(+). The signs of the vertical axis (α value) represent the optical rotation of the first-eluted enantiomers. Eluent: hexane–2-propanol (98:2, v/v). [a]The signs of the vertical axis (α value) represent the CD detection (254 nm) of the first-eluted enantiomer

2.5 *Other Synthetic Helical Polymers Showing Chiral Recognition*

An end-functionalized poly(4′-isocyanatobenzo-18-crown-6) (**57**) consists of achiral monomer units, but took a preferred-handed helical conformation induced by the optically active residue incorporated at the chain end as the initiator ("domino effect") [98], as supported by a rather intense CD induced by the chiral terminal group that appeared in the polymer backbone chromophore region. The polyisocyanate that

showed a chiral discrimination toward racemic amino acid derivatives, such as DL-**58** and the D-isomer, was enantioselectively extracted during the liquid–liquid extraction with **57**, whereas the corresponding unimer of **57** ($n = 1$) showed almost no enantioselectivity, indicating that a supramolecular helical array of achiral functional crown ether pendants with an excess screw-sense along the polymer backbone contributes to the present chiral recognition.

A stereoregular poly(phenylacetylene) bearing an analogous crown ether as the pendant (**59**) was quite sensitive to the chirality of the guest molecules, such as amino acids, and an almost one-handed helical conformation was induced in the entire polymer chain in the presence of 0.1 equiv. of L-Ala in acetonitrile [99]. The polymer showed an intense CD in the conjugated polymer backbone region even with 0.01 equiv. of L-Ala or 5% ee of Ala, indicating a remarkable chiral amplification with cooperative interactions in the pendant groups through noncovalent chiral interactions, and it could be applied to sense a tiny enantiomeric imbalance of specific guest molecules.



Poly(methyl methacrylate) (PMMA) constitutes a class of commodity plastics. When the polymer has a stereoregular syndiotactic (st) main-chain configuration, the st-PMMA forms a thermoreversible physical gel in aromatic solvents, such as toluene, due to a helical structure that possesses a sufficiently large cavity of ca. 1 nm, in which solvents are encapsulated [100]. Using an optically active alcohol, such as (*S*)- or (*R*)-1-phenylethanol (**60**) as the optically active additive in toluene, a preferred-handed helix can be induced in the st-PMMA backbone accompanied by gelation, and at the same time, fullerenes, such as C_{60}, C_{70}, and C_{84}, are encapsulated within its helical cavity to form a robust, processable peapod-like crystalline complex (Fig. 12a), as evidenced by the X-ray diffraction (XRD) profiles of the st-PMMA/C_{60} films and also by AFM and transmission electron microscopy (TEM) images of an st-PMMA/C_{60} film [101]. After complete removal of the optically active **60**, the st-PMMA–C_{60} complex gel showed vibrational circular dichroism (VCD) and CD signals in the PMMA IR and in the encapsulated C_{60} chromophore regions, respectively, although C_{60} itself is achiral, indicating that the macromolecular helicity of st-PMMA is memorized in the gel. The one-handed helical st-PMMA with a macromolecular helicity memory can recognize the size and chirality of fullerenes through an induced-fit mechanism. In fact, when an equimolar mixture of C_{60} and C_{70} in toluene was mixed with st-PMMA, C_{70} was

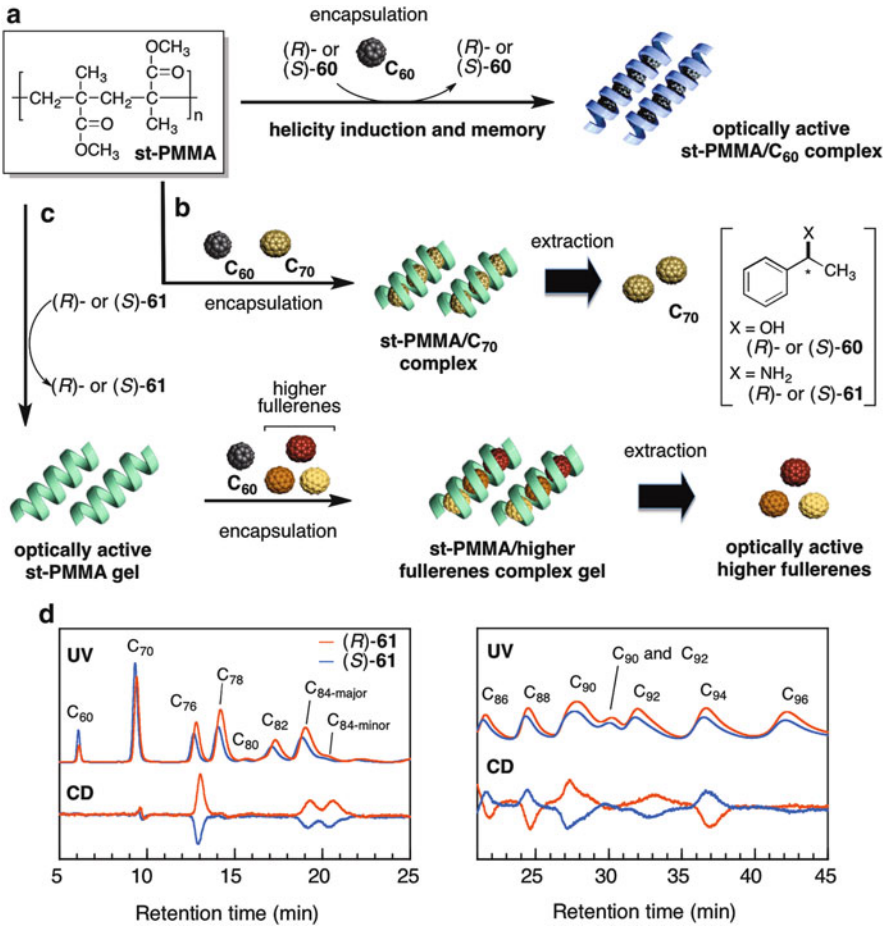


Fig. 12 (**a**) Schematic illustration of a helicity induction in st-PMMA in the presence of C_{60} with (*S*)- or (*R*)-**60**, and memory of the induced helicity after removal of **60**. (**b**) Schematic illustration of the preferential encapsulation of C_{70} over C_{60} by st-PMMA. (**c**) Selective extraction and resolution of higher fullerenes by helical st-PMMA with the induced helicity. (**d**) UV (356 nm, *top*) and CD (375 nm, *bottom*) detected HPLC chromatograms of the extracted fullerenes from carbon soot using optically active helical st-PMMAs prepared with (*R*)-**61** (*red lines*) and (*S*)-**61** (*blue lines*). (Reproduced with permission from [102]. Copyright 2010 American Chemical Society)

almost perfectly extracted with a selectivity of 99.8% (Fig. 12b) [102]. In addition, the optically active st-PMMA with an excess single-handed helix selectively extracted chiral higher fullerenes from carbon soot, and a series of optically active fullerenes (C_{76}, C_{84}, C_{86}, C_{88}, C_{90}, C_{92}, C_{94}, and C_{96}) were successfully extracted in one shot, as supported by the CD-detected HPLC chromatograms of the extract showing the apparent CDs (Fig. 12c,d) [102]. This strategy may provide a practical and valuable method for the size- and enantiomer-selective extraction of the elusive higher fullerenes.

3 Chiral Recognition Using Natural Helical Polymers and Their Derivatives

3.1 Polysaccharides

Polysaccharides, such as cellulose and amylose, are among the most abundant optically active biopolymers with perfect stereostructures, including stereogenic centers and tacticities. They have been used as chiral adsorbents since 1951 when Kotake resolved racemic amino acid derivatives by paper chromatography [103]. However, native cellulose and amylose are not effective as chiral packing materials for the separation of enantiomers, in particular for HPLC. Nowadays, more practically useful CSPs have been prepared by the modification of cellulose and amylose, and the tribenzoates of cellulose and trisphenylcarbamates of cellulose and amylose, having a one-handed helical conformation developed by Okamoto and the Daicel Corporation (Chart 1), have been recognized as the most widely used CSPs among more than 100 CSPs on the market, not only for analytical purposes, but also for the preparative scale separation of enantiomers including chiral drugs [5, 22–24, 27, 28, 104]. The detailed developments of these modified polysaccharide-based CSPs have been comprehensively reviewed elsewhere [5, 22–24, 27, 28, 104]. In this section, the most recent developments regarding the polysaccharide-based CSPs are briefly described.

The modified polysaccharide-based CSPs have been conventionally prepared by physically coating them on silica gel, while at present they are immobilized onto silica gel via chemical bonding, leading to ideal CSPs with a high durability against solvents, and some of them have been commercialized (Chart 2).

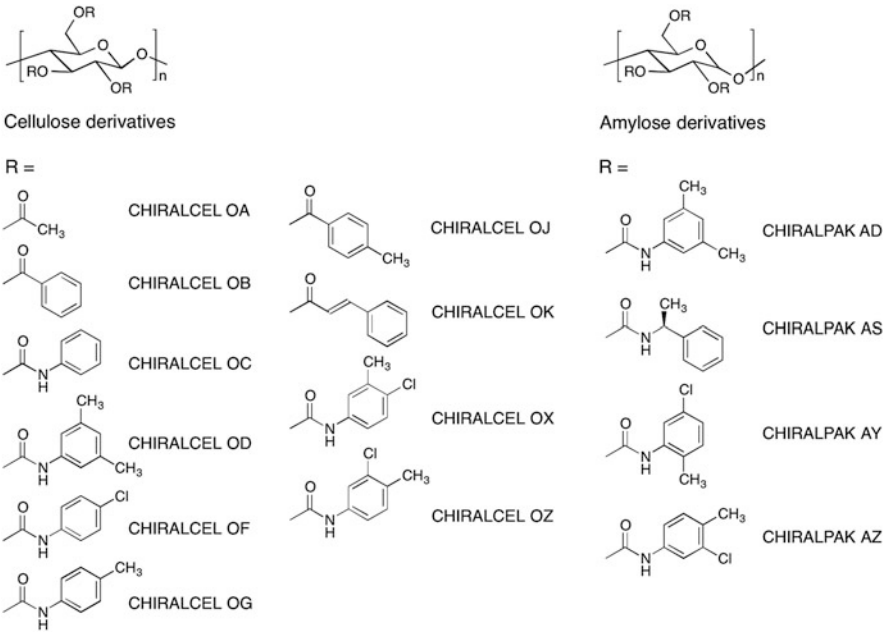


Chart 1

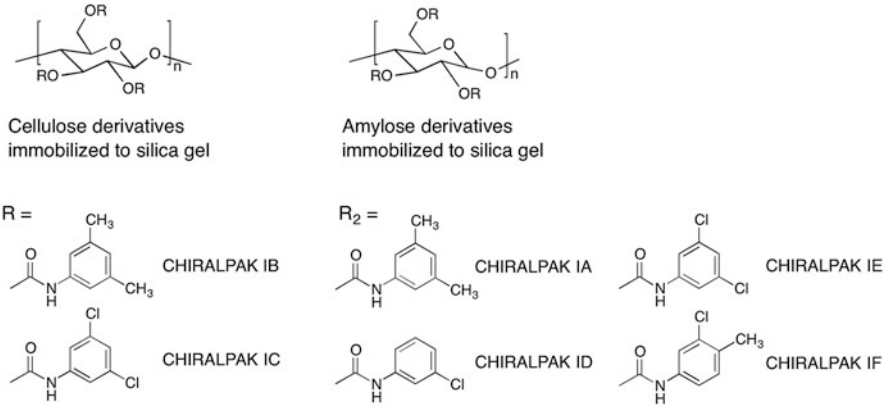


Chart 2

The first chemically bonded-type CSPs based on polysaccharide derivatives were reported in 1987; the cellulose trisphenylcarbamates were chemically linked with diisocyanates as a cross-linking reagent, which connect between the amino groups of the A-silica and the hydroxy groups of cellulose [105]. However, the resulting chemically bonded-type CSPs showed a low resolving ability, particularly when a large amount of cross-linking reagents was used, which may cause an alternation of the regular higher order structures, for example a helical conformation of the polysaccharide derivatives. Obviously, chemically bonding to silica gel at either end of the polysaccharide chains appears to be ideal, thus allowing the polysaccharides to maintain their ordered helical conformations even after immobilization.

To this end, amylose tris(3,5-dimethylphenylcarbamate) (CHIRALPAK AD) was chemically bonded to silica gel only at the reducing terminal residue of the amylose (Fig. 13a). Amylose, having the desired chain length with the reducing terminal residue, was readily prepared by the polymerization of the α-D-glucose 1-phosphate dipotassium salt with functionalized maltooligosaccharides using a phosphorylase isolated from potato. The amylose was then successfully bonded to silica gel only at the reducing terminus, followed by treatment with 3,5-dimethylphenyl isocyanate, to afford the chemically bonded-type CSP that exhibits an excellent resolving ability comparable to that of the coated-type CSP and high durability against solvents, such as THF and $CHCl_3$, and is commercially available (CHIRALPAK IA) [106]. Some racemates were more efficiently separated on this bonded-type CSP than on the coated-type CSP (CHIRALPAK AD) using $CHCl_3$ and THF as a component of the mobile phase (Fig. 14) [107].

This method is quite attractive, but only applicable to amylose derivatives, and hence a versatile method for immobilization of other polysaccharide derivatives has been developed. The radical copolymerization of vinyl residues introduced on the polysaccharide derivative with vinyl monomer-functionalized silica gel often afforded CSPs showing a slightly lower recognition ability [104, 108, 109]. To overcome this shortcoming, a polysaccharide derivative bearing a vinyl group was immobilized onto silica gel through radical copolymerization with a vinyl monomer

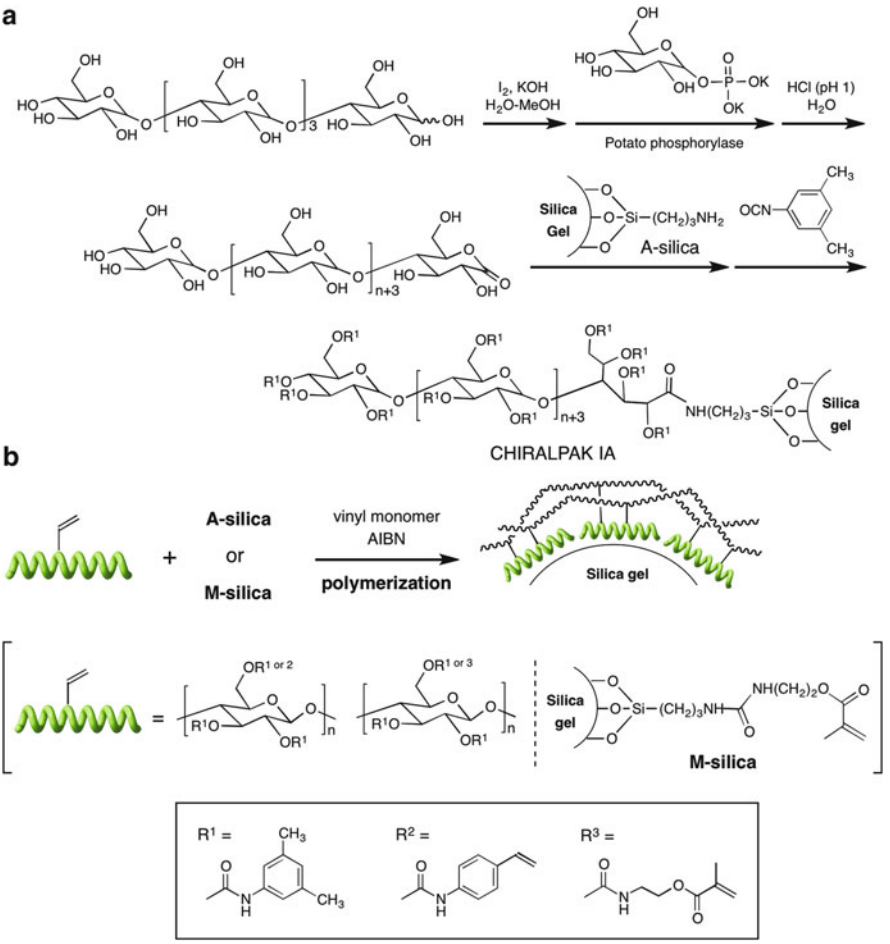


Fig. 13 (**a**) Immobilization of an amylose derivative onto silica gel only at the reducing terminal residue. (**b**) Immobilization of cellulose derivatives bearing a vinyl group onto silica gel by radical copolymerization with a vinyl monomer

[110–113]. After optimization of the immobilization conditions, such as the type and amount of the vinyl group introduced to the derivatives or the vinyl monomers, the chemically bonded-type cellulose-based CSPs showing a high recognition ability comparable to those of the coated ones were obtained (Fig. 13b).

Another efficient and more versatile immobilization method has been developed using a sol–gel condensation technique [114, 115]. The cellulose (**63**) and amylose (**64**) derivatives bearing 1−2% 3-(triethoxysilyl)propyl groups were coated on silica gel, which were then allowed to react with trimethylsilylchloride in the presence of water, resulting in the immobilization of the polysaccharides onto silica gel through intermolecular polycondensation with a low degree of cross-linking (Fig. 15). Therefore, the immobilized CSPs exhibited a high recognition ability similar to those of the conventional coated-type CSPs. This immobilization method

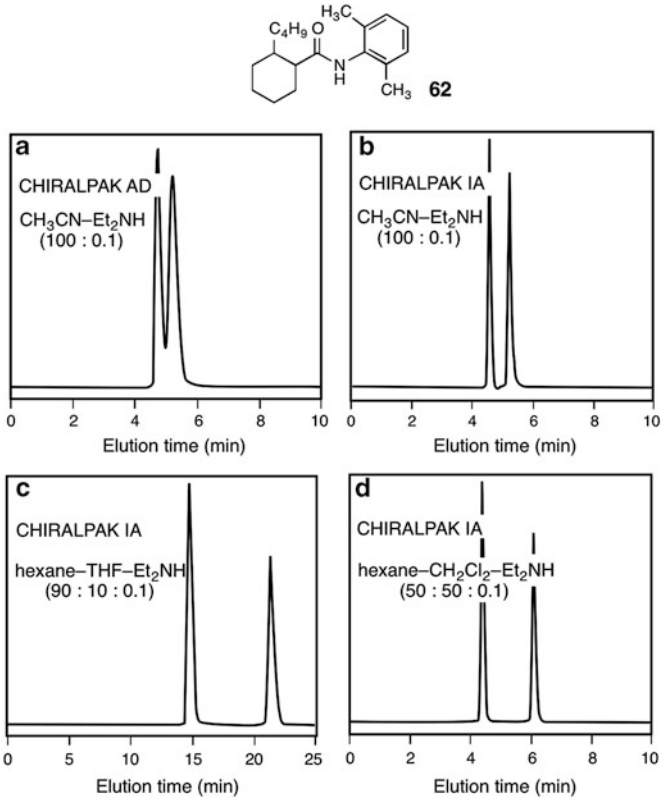


Fig. 14 Chromatograms for the resolution of **62** on CHIRALPAK AD (**a**) and CHIRALPAK IA (**b–d**). Eluent: CH_3CN–Et_2NH (100:0.1, v/v) (**a**,**b**), hexane–THF–Et_2NH (90:10:0.1, v/v) (**c**), hexane–CH_2Cl_2–Et_2NH (50:50:0.1, v/v) (**d**). (Reproduced with permission from [107]. Copyright 2005 Elsevier)

may be much better than others with respect to the simple and conventional processing and high immobilization efficiency, while retaining their high chiral recognition abilities, and can be used for other polysaccharide derivatives.

Amylose possesses a chiral hydrophobic cavity and is capable of forming inclusion complexes with specific small guest molecules [116] and polyrotaxanes with some polymers, such as carbon nanotubes that fit the cavity size in solution [117]. Recently, poly(*p*-phenylenevinylene) (PPV), an important π-conjugated luminescent polymer used as polymeric organic light-emitting diodes, was found to be encapsulated in amylose during the polymerization of its precursor monomer in an aqueous media, resulting in the luminescent amylose-PPV inclusion complex (APPV). The APPV is soluble in dimethyl sulfoxide (DMSO), and further modification of the hydroxy groups of the resulting amylose-PPV composite was possible [118]. Hence, a unique CSP composed of helical amylose tris(3,5-dimethylphenylcarbamate) (ADMPC) that encapsulates a rod-like PPV in its helical cavity (APPV-PC) has been prepared through a macromolecular reaction using the corresponding isocyanate (Fig. 16a) [119].

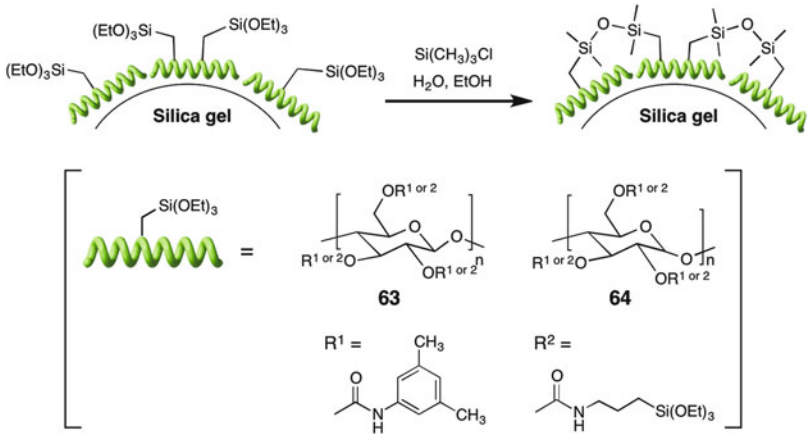


Fig. 15 Immobilization of polysaccharide derivatives bearing 3-(triethoxysilyl)propyl groups onto silica gel through the intermolecular polycondensation with a low degree of cross-linking

The CSP prepared from APPV-PC by coating on silica gel showed a remarkable chiral recognition ability for various racemates whose resolving ability was comparable to that of the commercially available amylose-based CSP (ADMPC, CHIRALPAK AD). Among the racemic compounds tested, the cyclic dibenzamide and dibenzanilide derivatives (**47** and **65**, respectively) were specifically resolved on APPV-PC ($\alpha = 3.17$ and 1.44, respectively) better than on ADMPC ($\alpha = 2.01$ and 1.08, respectively) as shown in Fig. 16b,c. The racemate **65** was partially separated on the ADMPC with the elution order of enantiomers such that the (+)-isomer eluted first followed by the (−)-isomer, whereas the reversed elution order was observed on the APPV-PC (Fig. 16c). The APPV-PC retains its helical inclusion structure after chemical modification of the hydroxy groups of the exterior amylose, and, therefore, its helical structure may be different from that of ADMPC, which results in the different chiral recognition abilities for several racemates.

3.2 Polypeptides and Nucleic Acids

Proteins and nucleic acids are fundamental to all living organisms and are optically active due to their inherent homo-chirality. Therefore it is quite natural to expect that proteins and nucleic acids may show chiral recognition abilities when used as CSPs. In fact, the first chiral separation of enantiomers using a protein-based CSP in liquid chromatography was reported in 1973 [120]. Since then, a variety of protein based-CSPs have been developed and some of them have been commercialized [29–31, 121]. Stereoselective monoclonal antibodies to D- and L-α-amino acids, raised against protein conjugates of *p*-amino-D- and L-phenylalanine, were immobilized on a chromatographic support, which could quite efficiently separate a

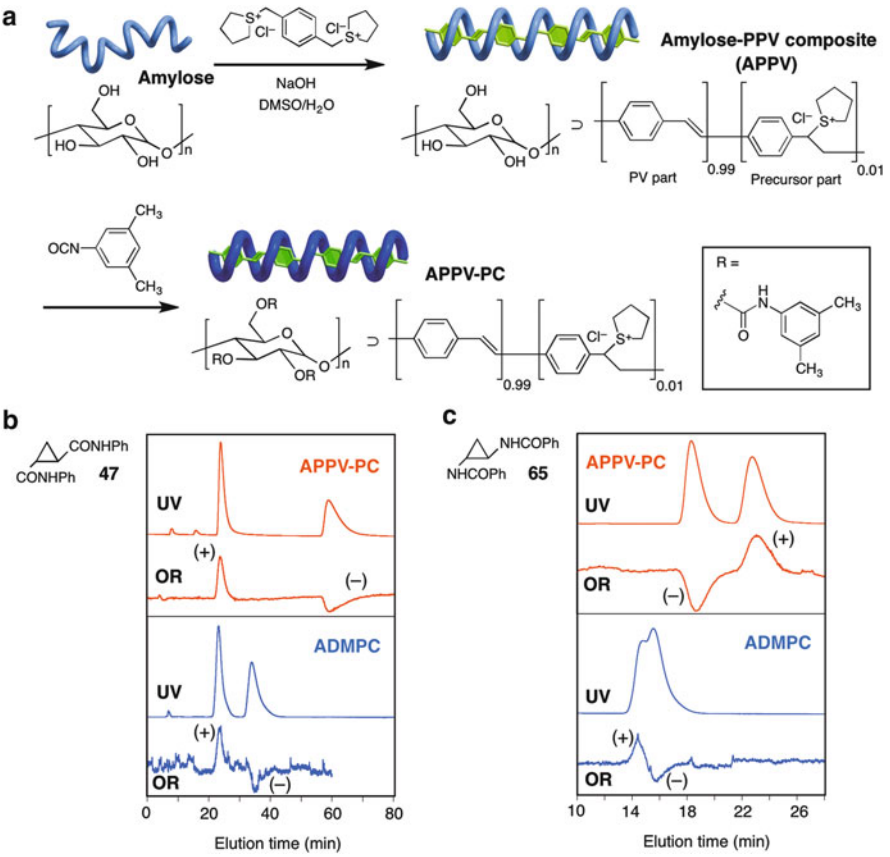


Fig. 16 (**a**) Schematic illustration of the synthesis of APPV and its derivative modified with 3,5-dimethylphenyl isocyanate (APPV-PC). The core PPV polymer contains approximately 1 mol% of the precursor units. (**b**,**c**) Chromatograms for the resolution of **47** (**b**) and **65** (**c**) on APPV-PC (column = 25 cm × 0.20 cm (i.d.), flow rate = 0.1 mL/min, *red lines*) and ADMPC (column = 25 cm × 0.46 cm (i.d.), flow rate = 0.5 mL/min, *blue lines*), respectively. Eluent: hexane-2-propanol (9:1, v/v). (Reproduced with permission from [119]. Copyright 2011 The Chemical Society of Japan)

number of amino acids into enantiomers with separation factors (α) up to 136 [122]. α-Helical polypeptides are a class of interesting synthetic helical polymers that also have potential as CSPs. Nevertheless, efficient CSPs with the same level of other helical polymer-based CSPs, including those based on helical poly(meth)acrylamides and polymethacrylates, and derivatized polysaccharides have not yet been developed. Initially, poly(L-glutamic acid) derivatives were immobilized on a cross-linked polystyrene gel to prepare the CSPs (**66a–c**) [123]. Among three CSPs, poly(N^5-benzyl-L-glutamine) with a right-handed helical conformation (**66c**) most effectively resolved racemic mandelic acid and the D-enantiomer was preferentially adsorbed. In contrast, poly(N^5-benzyl-D-glutamine)

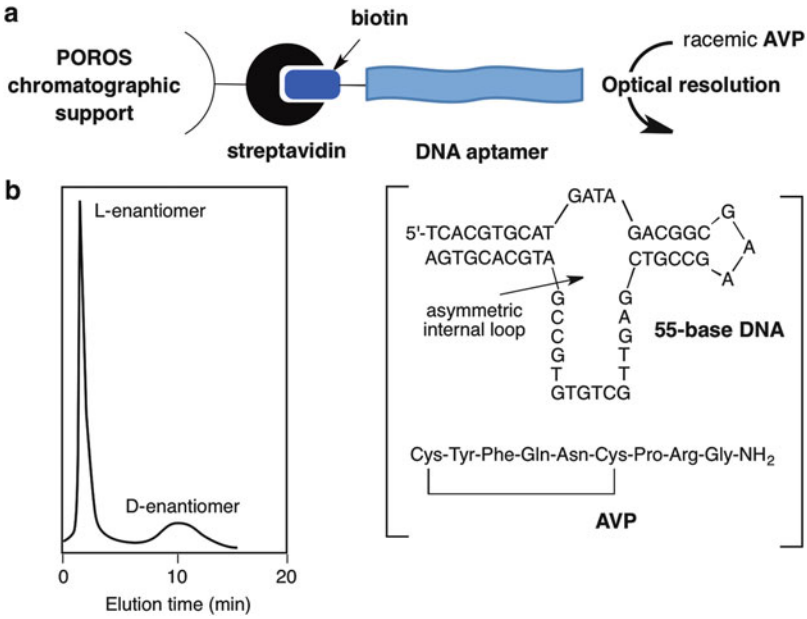
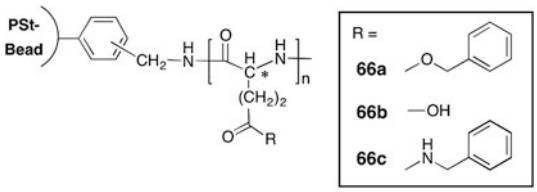


Fig. 17 (**a**) Schematic illustration of the immobilization of the DNA aptamer, and structures of the 55-base DNA aptamer and AVP. (**b**) Chromatographic resolution of racemic AVP on the DNA-based CSP. Eluent: 5 mM phosphate buffer containing 3 mM $MgCl_2$ (pH 6.0)

and poly(N^4-benzyl-L-asparagine), adopting the opposite left-handed helical conformation, showed an opposite enantioselectivity; the L-enantiomer preferentially adsorbed over the D-enantiomer, indicating an important effect of the helical structure on the chiral resolution. The dominant role of the helical structure and its handedness was also supported by the fact that the helical poly(N^5-benzyl-L-glutamine) (**66c**) with a longer main-chain (degree of polymerization (DP) = 18 or 11) exhibited a better separation factor ($\alpha = 1.08$) for mandelic acid than that of non-helical poly(N^5-benzyl-L-glutamine) with DP = 2 ($\alpha = 1.01$).



DNA and RNA aptamers have also been used as CSPs for HPLC [124–126]. For example, the 5′-biotinylated 55-base DNA aptamer, which is known to bind stereospecifically the (all-D)-enantiomer of an oligopeptide with a specific sequence (arginine-vasopressin, AVP), was immobilized on a streptavidin-bound chromatographic support through noncovalent biotin-streptavidin interactions (Fig. 17a). The resulting DNA-based CSP completely resolved a racemic mixture of

AVP into the enantiomers by HPLC (Fig. 17b) and the target D-peptide was more retained than the L-peptide [124]. This approach, including the use of RNA aptamers, has been extended to the separation of small chiral molecules, such as adenosine and tyrosinamide [125, 127].

4 Conclusion

As briefly described in this chapter, significant progress has been made in the synthesis of helical polymers over the past decade, which has significantly contributed to the development of novel polymer-based CSPs for efficiently differentiating enantiomers in HPLC. Apart from numerous synthetic variations in helical polymer syntheses, however, the rational design of synthetic helical polymers with a controlled helical sense from monomers via the polymerization process still remains a challenge. Among the commercially available CSPs, the cellulose- and amylose-based CSPs are the most frequently used CSPs and can resolve a wide range of racemates with a variety of functional groups on both analytical and preparative scales. Most importantly, the cellulose- and amylose-based CSPs belong to a class of helical polymers possessing a one-handed helical conformation after proper modification of the reactive hydroxy groups into esters and carbamates, which are also arranged into one-handed helical arrays along the polymer backbones, being responsible for their very high chiral recognition during diastereomeric interactions with each enantiomer. These structural features will provide a clue to the design and development of new helical polymers with a chiral resolving ability as high as those of the polysaccharide-based CSPs.

References

1. Natta G, Pino P, Corradini P, Danusso F, Mantica E, Mazzanti G, Moraglio G (1955) J Am Chem Soc 77:1708
2. Okamoto Y, Suzuki K, Ohta K, Hatada K, Yuki H (1979) J Am Chem Soc 101:4763
3. Blaschke G (1980) Angew Chem Int Ed 19:13
4. Maier NM, Franco P, Lindner W (2001) J Chromatogr A 906:3
5. Francotte ER (2001) J Chromatogr A 906:379
6. Andersson S, Allenmark SG (2002) J Biochem Biophys Methods 54:11
7. Roussel C, Rio AD, Pierrot-Sanders J, Piras P, Vanthuyne N (2004) J Chromatogr A 1037:311
8. Liu Y, Lantz AW, Armstrong DW (2004) J Liq Chromatogr Relat Technol 27:1121
9. Zhang Y, Wu DR, Wang-Iverson DB, Tymiak AA (2005) Drug Discov Today 10:571
10. Busch MA, Busch KW (2006) Chiral analysis. Elsevier, Amsterdam
11. Francotte E, Lindner W (2006) Chirality in drug research. Wiley-VCH Verlag GmbH & Co KGaA, Weinheim
12. Gübitz G, Schmid MG (2006) Mol Biotechnol 32:159
13. Subramanian G (2007) Chiral separation techniques: a practical approach. Wiley, Weinheim

14. Okamoto Y, Ikai T (2008) Chem Soc Rev 37:2593
15. Gasparrini F, Misiti D, Villani C (2001) J Chromatogr A 906:35
16. Ward TJ, Farris AB (2001) J Chromatogr A 906:73
17. Davankov VA (2003) J Chromatogr A 1000:891
18. Hyun MH (2005) Bull Korean Chem Soc 26:1153
19. Lämmerhofer M, Lindner W (2007) Adv Chromatogr 46:1
20. D'Acquarica I, Gasparrini F, Misiti D, Pierini M, Villani C (2008) Adv Chromatogr 46:109
21. Oguni K, Oda H, Ichida A (1995) J Chromatogr A 694:91
22. Yashima E, Yamamoto C, Okamoto Y (1998) Synlett 344
23. Okamoto Y, Yashima E (1998) Angew Chem Int Ed 37:1020
24. Yashima E (2001) J Chromatogr A 906:105
25. Tachibana K, Ohnishi A (2001) J Chromatogr A 906:127
26. Stringham RW (2006) Adv Chromatogr 44:257
27. Ikai T, Okamoto Y (2009) Chem Rev 109:6077
28. Okamoto Y, Ikai T, Shen J (2011) Israel J Chem 51:1096
29. Haginaka J (2001) J Chromatogr A 906:253
30. Millot MC (2003) J Chromatogr B 797:131
31. Haginaka J (2008) J Chromatogr B 875:12
32. Nakano T (2001) J Chromatogr A 906:205
33. Yamamoto C, Okamoto Y (2004) Bull Chem Soc Jpn 77:227
34. Blaschke G, Kraft HP, Fickentscher K, Kohler F (1979) Arzneimittel-Forschung/Drug Research 29:1640–1642
35. Müller GW (1997) ChemTech 27:21
36. Stinson SC (1994) Chem Eng News 72:38
37. Blaschke G, Bröker W, Fraenkel W (1986) Angew Chem Int Ed 25:830
38. Morioka K, Suito Y, Isobe Y, Habaue S, Okamoto Y (2003) J Polym Sci, Part A: Polym Chem 41:3354
39. Zhou H, Onimura K, Tsutsumi H, Oishi T (2001) Polym J 33:227
40. Onimura K, Zhang Y, Yagyu M, Oishi T (2004) J Polym Sci, Part A: Polym Chem 42:4682
41. Oishi T, Zhang Y, Fukushima T, Onimura K (2005) Polym J 37:453
42. Oishi T, Gao H, Nakamura T, Isobe Y, Onimura K (2007) Polym J 39:1047
43. Nakano T, Okamoto Y (2001) Chem Rev 101:4013
44. Okamoto Y, Honda S, Okamoto I, Yuki H, Murata S, Noyori R, Takaya H (1981) J Am Chem Soc 103:6971
45. Yuki H, Okamoto Y, Okamoto I (1980) J Am Chem Soc 102:6356
46. Kissener W, Vögtle F (1985) Angew Chem Int Ed 24:222
47. Muraoka T, Kinbara K, Kobayashi Y, Aida T (2003) J Am Chem Soc 125:5612
48. Okamoto Y, Yashima E, Hatada K, Mislow K (1984) J Org Chem 49:557
49. Dyker G, Stirner W, Henkel G, Schreiner PR (2008) Helv Chim Acta 91:904
50. Koumura N, Geertsema EM, van Gelder MB, Meetsma A, Feringa BL (2002) J Am Chem Soc 124:5037
51. Mateo Alonso A, Horcajada R, Groombridge HJ, Chudasama R, Motevalli M, Utley JHP, Wyatt PB (2005) Org Biomol Chem 3:2832
52. Shintani R, Moriya K, Hayashi T (2011) J Am Chem Soc 133:16440
53. Okamoto Y, Hatada K (1986) J Liq Chromatogr 9:369
54. Okamoto Y, Mohri H, Hatada K (1989) Polym J 21:439
55. Okamoto Y, Yashima E, Ishikura M, Hatada K (1987) Polym J 19:1183
56. Okamoto Y, Yashima E, Ishikura M, Hatada K (1988) Bull Chem Soc Jpn 61:255
57. Nakano T, Satoh Y, Okamoto Y (1998) Polym J 30:635
58. Okamoto Y, Mohri H, Nakamura M, Hatada K (1987) J Chem Soc Jpn 435
59. Okamoto Y, Mohri H, Nakano T, Hatada K (1989) J Am Chem Soc 111:5952
60. Nakano T, Shikisai Y, Okamoto Y (1996) Polym J 28:51
61. Nakano T, Satoh Y, Okamoto Y (2001) Macromolecules 34:2405

62. Haupt K, Mosbach K (2000) Chem Rev 100:2495
63. Wulff G (2002) Chem Rev 102:1
64. Yashima E, Maeda K, Nishimura T (2004) Chem Eur J 10:42
65. Shiotsuki M, Sanda F, Masuda T (2011) Polym Chem 2:1044
66. Yashima E, Maeda K (2008) Macromolecules 41:3
67. Yashima E, Maeda K, Furusho Y (2008) Acc Chem Res 41:1166
68. Liu J, Lam JWY, Tang BZ (2009) Chem Rev 109:5799
69. Yashima E, Huang S, Okamoto Y (1994) J Chem Soc, Chem Commun 1811
70. Yashima E, Matsushima T, Nimura T, Okamoto Y (1996) Korea Polym J 4:139
71. Marcelli T, Hiemstra H (2010) Synthesis 1229
72. Yeboah EMO, Yeboah SO, Singh GS (2011) Tetrahedron 67:1725
73. Tang Z, Iida H, Hu H-Y, Yashima E (2012) ACS Macro Lett 1:261
74. Naito Y, Tang Z, Iida H, Miyabe T, Yashima E (2012) Chem Lett 41:809
75. Miyake GM, Iida H, Hu H-Y, Tang Z, Chen EYX, Yashima E (2011) J Polym Sci, Part A: Polym Chem 49:5192
76. Zhang C, Liu F, Li Y, Shen X, Xu X, Sakai R, Satoh T, Kakuchi T, Okamoto Y (2013) J Polym Sci, Part A: Polym Chem 51:2271–2278
77. Aoki T, Kaneko T (2005) Polym J 37:717
78. Aoki T, Fukuda T, Shinohara KI, Kaneko T, Teraguchi M, Yagi M (2004) J Polym Sci, Part A: Polym Chem 42:4502
79. Hadano S, Teraguchi M, Kaneko T, Aoki T (2007) Chem Lett 36:220
80. Liu RY, Sanda F, Masuda T (2008) J Polym Sci, Part A: Polym Chem 46:4175
81. Sanda F, Fujii T, Tabei J, Shiotsuki M, Masuda T (2008) Macromol Chem Phys 209:112
82. Nolte RJM, Van Beijnen AJM, Drenth W (1974) J Am Chem Soc 96:5932
83. Yamagishi A, Tanaka I, Taniguchi M, Takahashi M (1994) J Chem Soc, Chem Commun 1113
84. Tsuchida A, Hasegawa T, Kobayashi K, Yamamoto C, Okamoto Y (2002) Bull Chem Soc Jpn 75:2681
85. Onitsuka K, Joh T, Takahashi S (1992) Angew Chem Int Ed 31:851
86. Takei F, Hayashi H, Onitsuka K, Kobayashi N, Takahashi S (2001) Angew Chem Int Ed 40:4092
87. Okoshi K, Nagai K, Kajitani T, Sakurai S-i, Yashima E (2008) Macromolecules 41:7752
88. Onouchi H, Okoshi K, Kajitani T, Sakurai S-i, Nagai K, Kumaki J, Onitsuka K, Yashima E (2008) J Am Chem Soc 130:229
89. Wu Z-Q, Nagai K, Banno M, Okoshi K, Onitsuka K, Yashima E (2009) J Am Chem Soc 131:6708
90. Tamura K, Miyabe T, Iida H, Yashima E (2011) Polym Chem 2:91
91. Miyabe T, Iida H, Banno M, Yamaguchi T, Yashima E (2011) Macromolecules 44:8687
92. Yashima E, Maeda K, Iida H, Furusho Y, Nagai K (2009) Chem Rev 109:6102
93. Yashima E, Maeda K, Okamoto Y (1999) Nature 399:449
94. Ishikawa M, Maeda K, Mitsutsuji Y, Yashima E (2004) J Am Chem Soc 126:732
95. Hase Y, Ishikawa M, Muraki R, Maeda K, Yashima E (2006) Macromolecules 39:6003
96. Hase Y, Nagai K, Iida H, Maeda K, Ochi N, Sawabe K, Sakajiri K, Okoshi K, Yashima E (2009) J Am Chem Soc 131:10719
97. Miyabe T, Iida H, Yashima E (2012) Chem Sci 3:863
98. Sakai R, Otsuka I, Satoh T, Kakuchi R, Kaga H, Kakuchi T (2006) J Polym Sci, Part A: Polym Chem 44:325
99. Nonokawa R, Yashima E (2003) J Am Chem Soc 125:1278
100. Kusuyama H, Yakase M, Higashihata Y, Tseng HT, Chatani Y, Tadokoro H (1982) Polymer 23:1256
101. Kawauchi T, Kumaki J, Kitaura A, Okoshi K, Kusanagi H, Kobayashi K, Sugai T, Shinohara H, Yashima E (2008) Angew Chem Int Ed 47:515

102. Kawauchi T, Kitaura A, Kawauchi M, Takeichi T, Kumaki J, Iida H, Yashima E (2010) J Am Chem Soc 132:12191
103. Kotake M, Sakan T, Nakamura N, Senoh S (1951) J Am Chem Soc 73:2973
104. Franco P, Senso A, Oliveros L, Minguillón C (2001) J Chromatogr A 906:155
105. Okamoto Y, Aburatani R, Miura S-I, Hatada K (1987) J Liq Chromatogr 10:1613
106. Enomoto N, Furukawa S, Ogasawara Y, Akano H, Kawamura Y, Yashima E, Okamoto Y (1996) Anal Chem 68:2798
107. Zhang T, Kientzy C, Franco P, Ohnishi A, Kagamihara Y, Kurosawa H (2005) J Chromatogr A 1075:65
108. Oliveros L, Lopez P, Minguillon C, Franco P (1995) J Liq Chromatogr 18:1521
109. Kimata K, Tsuboi R, Hosoya K, Tanaka N (1993) Anal Methods Instrum 1:23
110. Kubota T, Kusano T, Yamamoto C, Yashima E, Okamoto Y (2001) Chem Lett 724
111. Kubota T, Yamamoto C, Okamoto Y (2003) J Polym Sci, Part A: Polym Chem 41:3703
112. Kubota T, Yamamoto C, Okamoto Y (2004) J Polym Sci, Part A: Polym Chem 42:4704
113. Chen X-M, Yamamoto C, Okamoto Y (2006) J Chromatogr A 1104:62
114. Ikai T, Yamamoto C, Kamigaito M, Okamoto Y (2007) J Chromatogr A 1157:151
115. Ikai T, Yamamoto C, Kamigaito M, Okamoto Y (2006) Chem Lett 35:1250
116. Kubik S, Holler O, Steinert A, Tolksdorf M, Wulff G (1995) Macromol Symp 99:93
117. Star A, Steuerman DW, Heath JR, Stoddart JF (2002) Angew Chem Int Ed 41:2508
118. Ikeda M, Furusho Y, Okoshi K, Tanahara S, Maeda K, Nishino S, Mori T, Yashima E (2006) Angew Chem Int Ed 45:6491
119. Tamura K, Sam NSM, Ikai T, Okamoto Y, Yashima E (2011) Bull Chem Soc Jpn 84:741
120. Stewart KK, Doherty RF (1973) Proc Natl Acad Sci USA 70:2850
121. Allenmark SG, Andersson S (1994) J Chromatogr A 666:167
122. Hofstetter O, Lindstorm H, Hofstetter H (2002) Anal Chem 74:2119
123. Kiniwa H, Nishikaji T, Ogata N (1987) J Polym Sci, Part A: Polym Chem 25:2689
124. Michaud M, Jourdan E, Villet A, Ravel A, Grosset C, Peyrin E (2003) J Am Chem Soc 125:8672
125. Brumbt A, Ravelet C, Grosset C, Ravel A, Villet A, Peyrin E (2005) Anal Chem 77:1993
126. Ravelet C, Peyrin E (2006) J Sep Sci 29:1322
127. Michaud M, Jourdan E, Ravelet C, Villet A, Ravel A, Grosset C, Peyrin E (2004) Anal Chem 76:1015