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Helicity induction and memory effect in poly(biphenylylacetylene)s bearing various functional groups and their use as switchable chiral stationary phases for HPLC

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A series of poly(biphenylylacetylene)s (PBPAs) bearing various functional groups at the 4'-position of the biphenyl pendants was synthesized and the effects of the pendant functional groups on their macromolecular helicity induction and subsequent memory behaviors as well as their chiral recognition abilities as chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC) were investigated. Both the macromolecular helicity and the pendant axial chirality of the PBPAs bearing ester, acyloxy or carbamate groups induced by noncovalent interactions with an optically active alcohol were more stably memorized compared to those induced in the PBPA bearing ether pendant groups when the optically active alcohol was completely removed. The PBPAs with a macromolecular helicity memory showed different chiral recognition abilities toward various racemates depending on the difference in the achiral pendant functional groups when used as CSPs for HPLC under normal phase conditions. The PBPA carrying carbamate pendants exhibited an excellent chiral recognition ability and resolved many racemates including axially chiral compounds and metal acetylacetonate complexes. Since the obtained PBPAs are soluble in the chiral alcohol, it was almost impossible to switch their enantioselectivities by treatment of the coated-type CSPs with a solution of the chiral alcohol in the column. By immobilizing the PBPA chains onto silica gel, the reversible control of the macromolecular helicity in the column could be achieved through alternative column treatment with a solution containing the (*R*)- or (*S*)-enantiomer of the chiral alcohol, thereby enabling the repeated switching of the enantiomer elution order.

Introduction

It is widely recognized that a pair of enantiomers often show significantly different physiological properties. Therefore, in various fields treating chiral compounds, the enantiomeric assay of chiral compounds as well as the acquisition of enantiomerically pure isomers have become very important issues.[1-9] Among the various methods, the enantioseparation by high-performance liquid chromatography (HPLC) using chiral stationary phases (CSPs) is considered to be one of the most popular and powerful ways for both analytical and preparative separations. Therefore, a number of CSPs consisting of a variety of optically active small molecules and polymers has been developed.[10-19] Since the successful application of a one-handed helical poly(triphenylmethyl methacrylate) (PTrMA) prepared by the helix-sense-selective polymerization[20-23] and helical polysaccharide derivatives[24-32] as a CSP for HPLC, synthetic helical polymers with a controlled helix-sense have attracted much attention as a promising chiral packing material for a practically useful CSP because of their highly-ordered helical structures being essential for their high chiral recognition abilities.

For the separation of enantiomers by chiral HPLC, the elution order of enantiomers is one of the key factors to obtain an efficient and desirable resolution result.[33-36] For the preparative scale separation, a target enantiomer with a higher optical purity can be obtained if it is eluted first because the peak of the second-eluting enantiomer is often overlapped with that of the first-eluting one. For analytical scale separation, the first elution of the minor enantiomer is generally desirable, leading to improvements in both the detection limit and the accuracy of the quantification.[37,38] In order to switch the elution order of the enantiomers during chiral HPLC under identical chromatographic conditions, the use of CSPs composed of enantiomeric chiral materials is required.

We previously reported that a poly(biphenylylacetylene) (PBPA) derivative possessing two methoxymethoxy (OMOM) groups at the 2- and 2'-positions and a *n*-dodecyloxy group at the 4'-position of the pendant biphenyl group (poly-**1a**) folds

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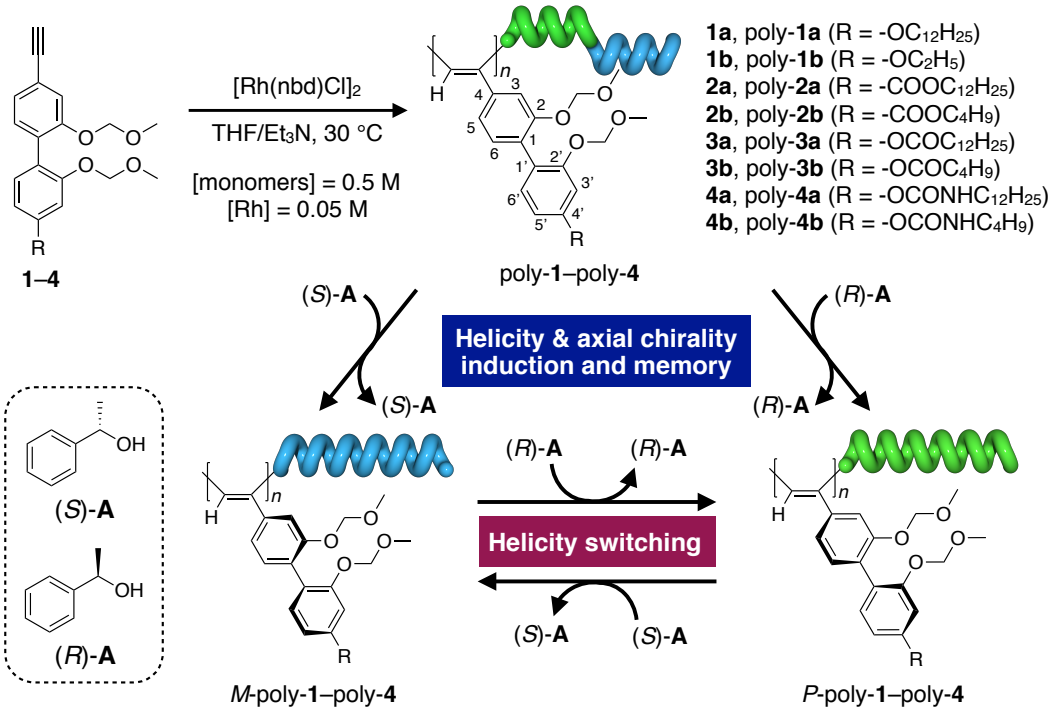
†Electronic Supplementary Information (ESI) available: Experimental details, CD intensity changes of poly-**1**–**4** in toluene/(*R*)-**A** or THF/(*R*)-**A** (80/20, v/v) with time, CD titration curves of poly-**1b** and poly-**2**–**4** with (*R*)-**A**, CD and absorption spectra of poly-**1b**–**4b** with (*R*)- and (*S*)-**A** in toluene or THF and those of the corresponding isolated polymers, ^{1}H NMR spectra of poly-**2** with (*R*)-**A** and the isolated poly-**2**, CD intensity changes of the isolated poly-**1b**–**3b** with time, VCD spectra of the isolated poly-**2a**–**4a**, CD and absorption spectra of the recovered poly-**2b** and poly-**3b** from silica surface and poly(**2b**-*co*-**5**), ^{1}H and ^{13}C NMR spectra of **5**, **1b**, **3** and **4**. See DOI: 10.1039/x0xx00000x



Scheme 1 Schematic illustration of synthesis of PBPAs bearing various functional groups at the 4'-position of the biphenyl pendants (poly-**1**–poly-**4**) and helicity induction, helicity switching and memory of the macromolecular helicity together with its axial chirality at the pendants of poly-**1**–poly-**4** through noncovalent interactions with a chiral alcohol (*S*)- or (*R*)-**A**.

into a right- or left-handed helical conformation upon interaction with (*R*)- or (*S*)-1-phenylethanol ((*R*)- or (*S*)-**A**) both in solution and in the solid state, respectively, accompanied by a twist-sense bias in the biphenyl pendants (Scheme 1).[39] Interestingly, the induced macromolecular helicity together with the axial chirality in the biphenyl pendants can be maintained, that is, memorized after complete removal of the chiral alcohol. By taking advantage of this unique helicity induction and subsequent memory effect of poly-**1a**, we successfully developed the first switchable CSP for HPLC using poly-**1a** as a chiral packing material which allows us to control and switch the elution order of the enantiomers by treatment with (*R*)- or (*S*)-**A** in the column (Scheme 1). However, the chiral recognition ability of the poly-**1a**-based CSP was not satisfactory mostly due to the lack of functional groups capable of effectively interacting with the enantiomers.

In fact, a PBPA derivative carrying a butoxycarbonyl group instead of a *n*-dodecyloxy group at the 4'-position of the biphenyl pendant group (poly-**2b**) was found to show a chiral recognition toward various racemic compounds better than poly-**1a** when used as a CSP for HPLC prepared by coating poly-**2b** onto silica gel (coated-type CSP) in the same way as the poly-**1a**-based CSP, while maintaining its macromolecular helicity memory effect (Scheme 1).[40] Moreover, the stability of the helicity memory of poly-**2b** was significantly improved compared to that of poly-**1a**.[40] We anticipated that further improvements of the enantioseparation capability as well as the helicity memory stability would be possible by introducing other functional groups at the 4'-position of the biphenyl pendants of the PBPAs, leading to the development of more efficient switchable CSPs. However, unlike the poly-**1a,** a switchable CSP could not be achieved using a poly-**2b**-based coated-type CSP because poly-**2b** was soluble in a solution containing (*S*)- or (*R*)-**A**, therefore, poly-**2b** was gradually removed from the column by passing a solution of (*S*)- or (*R*)-**A** through the column during the helicity induction and inversion processes.[40,41] To overcome this problem, the development of a facile method to immobilize the PBPAs onto silica gel through chemical bonding (immobilized-type CSP) is strongly desired.[25-28,42]

In this study, we synthesized a series of PBPAs bearing various functional groups at the 4'-position of the biphenyl pendants, such as alkyl ether (poly-**1a**[39] and poly-**1b**), alkoxycarbonyl (ester) (poly-**2a**[41] and poly-**2b**[40,41]), acyloxy (poly-**3a** and poly-**3b**) and carbamoyloxy (carbamate) (poly-**4a** and poly-**4b**) groups with long (poly-**1a**–poly-**4a**) or short (poly-**1b**–poly-**4b**) alkyl chains, and systematically investigated the effects of the functional groups on the helicity induction through noncovalent bonding interactions with (*S*)- or (*R*)-**A** followed by the memory of the induced helicity and their chiral recognition abilities as coated-type and/or immobilized-type CSPs for HPLC (Scheme 1). PBPAs with short ethyl (poly-**1b**) and *n*-butyl chains (poly-**2b**–poly-**4b**) were synthesized in order to prepare coated-type CSPs for HPLC enantioseparation under the normal-phase conditions with a *n*-hexane–2-propanol mixture as the eluent, in which the PBPAs with a long *n*-dodecyl chain (poly-**1a**–poly-**4a**) are soluble and cannot be used as a coated-type CSP for evaluating their chiral resolving abilities toward various racemates.

Results and discussion

Synthesis of Poly(biphenylylacetylene)s Bearing Various Functional Groups

Novel biphenylylacetylene monomers (**1b**, **3a**, **3b**, **4a** and **4b**) were synthesized as outlined in Scheme S1, then polymerized with a rhodium catalyst ($[Rh(nbd)Cl]_2$, nbd: norbornadiene) in THF containing triethylamine (Et_3N) at 30 °C according to the previously reported method (Scheme 1),[39-41] producing high molecular weight polymers (poly-**1b**, poly-**3a**, poly-**3b**, poly-**4a** and poly-**4b**, respectively) (the number-average molecular weight (M_n) > 3.7×10^5) in high yields (> 94%) (Table 1). The stereoregularities of these polymers were estimated to be highly *cis-transoidal* based on their ^{1}H NMR spectra, showing sharp singlets centered at *ca.* 6 ppm due to the main chain protons (ESI Fig. S48–S52†).[43-45] For comparison, the polymerization results of **1a**,[39] **2a**[41] and **2b**[40] are also shown in Table 1. Among the eight PBPAs, all the polymers, except for poly-**4b**, are soluble in toluene. Therefore, the helicity induction and memory experiments were performed in toluene, while THF was used as a solvent for poly-**4b**.

Helicity Induction and Memory of Macromolecular Helicity

The effects of the functional groups of the PBPAs bearing a long *n*-dodecyl chain (poly-**1a**–poly-**4a**) on the helicity induction with (*S*)- and (*R*)-**A** followed by the memory of the induced helicity were first investigated using circular dichroism (CD) spectroscopy. As shown in Fig. 1, poly-**2a**–poly-**4a** showed intense Cotton effects with mirror images of each other in the polymer backbone regions in the presence of (*R*)- and (*S*)-**A** in toluene (toluene/**A** = 80/20 (v/v)) at 25 °C (i and iv in Fig. 1a–c). The observed induced CD (ICD) spectral patterns of poly-**2a**–poly-**4a** as well as that of poly-**1a**[39] in the absorption regions of the polymer backbones are quite similar to each other, indicating that the helical structures of poly-**1a**–poly-**4a** induced by the chiral alcohol **A** are almost identical and independent of the pendant groups introduced at the 4'-position of the biphenyl pendants.

The ICD intensities of poly-**1a**–poly-**4a** gradually increased with time in toluene/(*R*)-**A** (80/20, v/v) at 25 °C and reached a near-plateau value within 1 h (poly-**1a**)[39] and after *ca.* 4 (poly-**4a**) and *ca.*12 h (poly-**2a** and poly-**3a**) (ESI Fig. S1, S3, S5 and S7†). The observed one-handed helix forming rate of the PBPAs tended to decrease in the following order: poly-**1a** > poly-**4a** > poly-**2a**, poly-**3a**, which may be related to the rotational barriers of the biphenyl pendants with an axial chirality. The preferred-handed macromolecular helicity induced in the PBPAs is likely coupled or synchronized with the axial chirality induction with a twist-sense bias upon interaction with (*R*)-**A**,[39] which could be influenced by the polarity of the 4'-functional groups on the biphenyl pendants. The CD titration experiments using (*R*)-**A** revealed that the ICD intensities of poly-**1a**–poly-**4a** reached an almost maximum value in the presence of *ca.* 10–20 vol % of (*R*)-**A** in toluene (ESI Fig. S2, S4, S6 and S8†). The ICD intensities of poly-**2a**–poly-**4a** also slightly increased at –10 °C in toluene/(*R*)-**A** (80/20, v/v) (ii in Fig. 1a–c).

Table 1 Polymerization results of **1**–**4** with $[Rh(nbd)Cl]_2$ in THF/Et_3N at 30 °C for 3 h[a]

Run	Monomer	Sample code	Yield (%)[b]	$M_n \times 10^5$ [c]	M_w/M_n [c]
1[d]	**1a**	poly-**1a**	98	4.9	1.8
2	**1b**	poly-**1b**	96	3.7	1.4
3[e]	**2a**	poly-**2a**	96	8.5	1.9
4[f]	**2b**	poly-**2b**	96	9.4	1.7
5	**3a**	poly-**3a**	96	8.5	1.9
6	**3b**	poly-**3b**	99	9.5	2.1
7	**4a**	poly-**4a**	96	5.2	1.9
8	**4b**	poly-**4b**	94	7.2	1.7

[a][Monomer] = 0.5 M, $[Rh(nbd)Cl]_2$ = 0.005 M. [b]Methanol insoluble part. [c]Determined by size exclusion chromatography (SEC) (polystyrene standards) with THF as the eluent. [d]Cited from ref. 39. [e]Cited from ref. 41. [f]Cited from ref. 40

As previously reported, the macromolecular helicities of poly-**1a** and poly-**2b** induced by (*S*)- or (*R*)-**A** were retained (memorized) after complete removal of the chiral **A** and the resulting static helicity memory of poly-**2b** was more stable than that of poly-**1a**.[39,40] We then investigated the effect of the substituents at the 4'-position of the biphenyl pendants of poly-**2a**–poly-**4a** on the stabilities of their helical conformations with a helicity memory. To this end, the optically active helical poly-**2a**–poly-**4a** induced by (*R*)-**A** in toluene (80/20, v/v) at 25 °C for 48 h were isolated by precipitation into methanol to completely remove (*R*)-**A** as confirmed by ^{1}H NMR measurements (see ESI Fig. S21 and S22† for typical ^{1}H NMR spectra). The CD spectra of the isolated poly-**2a**–poly-**4a** measured in toluene at –10 °C were nearly identical to those before isolation (ii and iii in Fig. 1a–c), indicating the complete static memory of the helicity that turned out to be possible for the poly-**2a**–poly-**4a** bearing different functional substituents at the 4'-position of the biphenyl pendants.

The stabilities of poly-**1a**–poly-**4a** with a helicity memory were investigated by following the CD intensity changes of the second Cotton effect ($\Delta\varepsilon_{2nd}$) with time in toluene at 25 °C (ESI Fig. S23†). The memory of the helical PBPAs bearing polar ester (poly-**2a**), acyloxy (poly-**3a**) or carbamate substituents on the biphenyl pendants (poly-**4a**) was much more stable than that of the previously reported poly-**1a** bearing ether groups; the half-life periods ($t_{1/2}$) increased in the following order: poly-**1a** (< 10 min) < poly-**4a** (2 h) < poly-**2a** (3 h) < poly-**3a** (5 h), which is mostly consistent with the reverse order of the one-handed helix forming rates (see above). The polar functional groups at the 4'-position of the biphenyl pendants of poly-**2a**–poly-**4a** seem to contribute to stabilizing the macromolecular helicity memory probably due to the intramolecular dipole-dipole and/or hydrogen bonding interactions between the neighboring polar functional groups.

The stability of the helicity memory of PBPAs was anticipated to be affected by the solvents, more specifically,

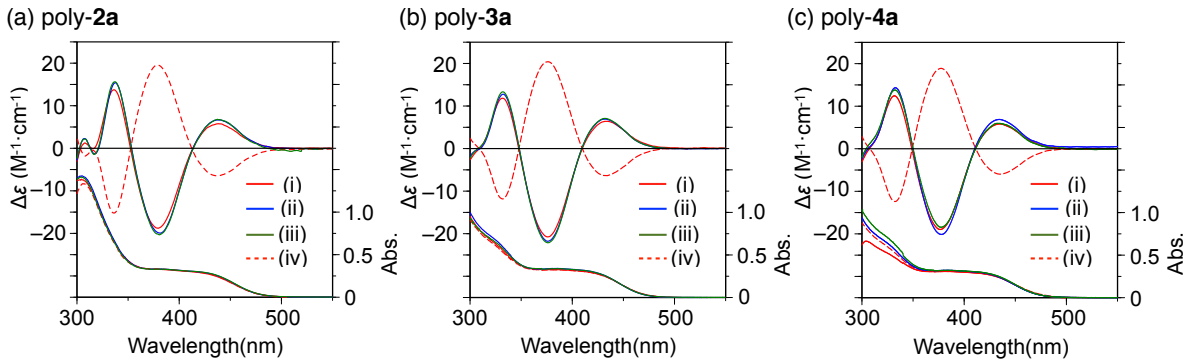


Fig. 1 CD and absorption spectra of poly-**2a** (a), poly-**3a** (b) and poly-**4a** (c) in toluene with (*R*)-**A** (toluene/(*R*)-**A** = 80/20, v/v) at 25 °C (i) and –10 °C (ii) after standing at 25 °C for 48 h, and the isolated polymers from ii (iii) measured in toluene at –10 °C. CD and absorption spectra of poly-**2a**–poly-**4a** in toluene with (*S*)-**A** (toluene/(*S*)-**A** = 80/20, v/v) at 25 °C (iv) after standing at 25 °C for 48 h are also shown. [polymer] = 1.0 mM.

solvent polarity.[39] In fact, the CD intensity of the isolated poly-**2a** decreased faster in polar solvents, such as THF and dichloromethane, than in nonpolar solvents, such as methylcyclohexane (MCH) and toluene at –10 °C (ESI Fig. S24†). It should be noted that the CD intensity of the memorized poly-**2a** remained almost unchanged in MCH at –10 °C after 72 h (ESI Fig. S24†) and also in the solid state at 25 °C at least for one month (ESI Fig. S27†). In chloroform and dichloromethane, however, the poly-**2a** lost its helical memory within a few minutes at 25°C (ESI Fig. S26†), which implies that we can instantly erase the helicity memory in these solvents.

The vibrational circular dichroism (VCD) spectra of the helicity-memorized poly-**2a**–poly-**4a** induced by (*R*)- and (*S*)-**A** measured at –10 °C exhibited intense split-type VCD signals which were mirror images of each other, for example, those at around 1,000 cm^{-1} due to the C–O–C stretching bands of the OMOM groups at the 2,2'-positions of the biphenyl pendants (ESI Fig. S28–S30†). These VCD results indicated that the axially chiral biphenyl pendants were also twisted into one direction upon interactions with (*R*)- and (*S*)-**A** as observed for poly-**1a**.[39] In addition, these polymers also showed virtually mirror-image VCD signals due to the C=O stretching vibrations of the ester (alkoxycarbonyl (poly-**2a**) or acyloxy (poly-**3a**)) or carbamate (poly-**4a**) groups in the pendants, indicating that the pendant ester and carbamate groups at the 4'-position of the biphenyl groups are arranged in a one-handed helical array along the helical polymer backbones with the helicity memory, thereby showing the VCD signals in the achiral pendant IR regions.

Prior to preparing coated-type and immobilized-type CSPs for evaluating the chiral recognition abilities of poly-**1b**–poly-**4b** with a macromolecular helicity memory by HPLC, the helicity induction and memory of poly-**1b**–poly-**4b** carrying a short alkyl chain and their stabilities of the helicity memory were investigated in the same way for poly-**1a**–poly-**4a** except for poly-**4b** because poly-**4b** was not soluble in the (*R*)-**A**/toluene mixtures, and instead, (*R*)-**A**/THF mixtures were used. As shown in ESI Fig. S9–S20 and S25†, poly-**1b**–poly-**3b** showed almost similar helicity induction and memory behaviors, although the ICD intensities of poly-**1b** and poly-**3b** induced by (*R*)- and (*S*)-**A** in toluene (80/20, v/v) at 25 °C after 48 h and hence those after isolation were slightly lower than those of the poly-**1a** and poly-**3a**, respectively. The reason for this is not clear at present, but the difference in the pendant alkyl chain length may be a concern. Poly-**4b** showed ICDs as intense as those of poly-**1a** in THF in the presence of (*R*)- and (*S*)-**A** (60/40, v/v) after 48 h at 25 °C and the induced helicity of poly-**4b** was successfully memorized (ESI Fig. S12, S19 and S20†).

Chiral recognition abilities of coated-type PBPA-based CSPs for HPLC

The chiral recognition abilities of poly-**1b**–poly-**4b** with a macromolecular helicity memory were first evaluated as coated-type CSPs, which were prepared as follows. Poly-**1b**–poly-**3b** and poly-**4b** dissolved in a mixture of toluene/(*R*)-**A** (80/20, v/v) and THF/(*R*)-**A** (60/40, v/v), respectively, were stored at 25 °C for 48 h to completely induce an almost one-handed helical conformation as already described. The polymer solutions were then coated on macroporous silica gel, which were packed into a stainless-steel column (25 x 0.20 cm (i.d.)).[46,47] The CSPs were then thoroughly washed by passing a *n*-hexane–2-propanol (97/3, v/v) mixture through the columns to completely remove (*R*)-**A**. During the helicity induction in poly-**1b**–poly-**4b** followed by the coated-type CSPs' preparation process, the induced macromolecular helicities of the polymers were almost retained as supported by the facts that the ICD signals of poly-**2b** and poly-**3b** recovered from the CSPs in toluene at –10 °C remained with a slight decrease in their intensities compared to those before coating onto silica gel (ESI Fig. S31 and S32†).

The enantioseparation results of the twelve racemates **B**–**M** on poly-**1b**–poly-**4b**-based coated-type CSPs are summarized in Table 2. As the eluent, a *n*-hexane–2-propanol mixture (97/3, v/v) was used throughout the course of the HPLC enantioseparation on the coated-type CSPs because the polymers are not soluble in the eluent. Fig. 2 shows the chromatograms for the resolution of racemic **D** and **E** on the poly-**3b**- and poly-**4b**-based CSPs, respectively, detected by UV and CD detectors. As shown in Fig. 2b, the (+)- and (–)-enantiomers were eluted at the retention times of t_1 and t_2, respectively, showing complete baseline separation. Based on

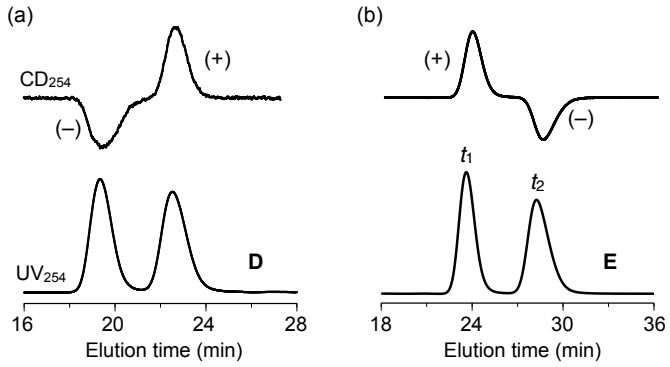


Fig. 2 HPLC chromatograms for the resolutions of **D** (a) and **E** (b) on poly-**3b**- and poly-**4b**-based coated-type CSPs, respectively, prepared by the interaction with (*R*)-**A**. Eluent: *n*-hexane–2-propanol (97/3, v/v).

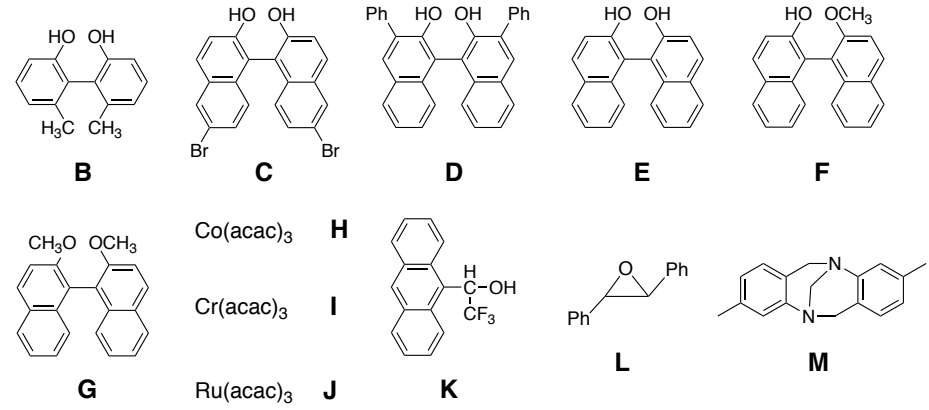
the hold-up time (t_0) (3.86 min), chromatographic parameters, the retention factors, k_1 [= (t_1–t_0)/t_0] and k_2 [=(t_2–t_0)/t_0], and separation factor α (= k_2/k_1) were determined to be 5.12, 6.32 and 1.23, respectively.

Among the four CSPs, the poly-**4b**-based coated-type CSP showed a better chiral recognition than the other CSPs and separated eight racemates among the twelve tested racemates, while poly-**2b** and poly-**3b** resolved six and three racemates, respectively. In contrast, poly-**1b** bearing *n*-butyl ether substituents could not resolve all the racemates.[48] The carbamate-bound poly-**4b** resolved well the axially chiral biaryl compounds with one or two hydroxy groups **B**–**F** as well as the chiral metal tris(acetylacetonato)s **H**–**J** and exhibited an excellent chiral recognition ability for the sterically hindered, axially chiral diol **D** with bulky substituents around the hydroxy groups; its α value reached over 4.0. Interestingly, for the structurally similar 1,1'-binaphthyl derivatives **E**–**G**, interaction in retention (k_1) and the enantioselectivity (α) toward the poly-**4b** remarkably decreased with the decreasing number of hydroxy groups, and the racemate **G** with two methoxy groups could not be resolved at all on poly-**4b**. The same tendency was observed when poly-**2b** was used as the coated-type CSP (Table 2).[40] These results suggest that hydrogen bonding interactions between the hydroxy groups of the binaphthyl racemates **D**–**F** and the pendant carbamate groups of poly-**4b** or the pendant oxycarbonyl groups of poly-**2b** are mostly the main force for their retentions and separation, and the simultaneous hydrogen bonds through the two hydroxy groups may contribute to a more effective resolution.

Table 2 Resolution results of racemates **B**–**M** on poly-**1b**–poly-**4b**-based coated-type CSPs[a]

CSP	poly-**1b**		poly-**2b**[b]		poly-**3b**		poly-**4b**	
Racemate	k_1	α	k_1	α	k_1	α	k_1	α
B	0.54	1.0	1.04	ca.1 (+)	0.75	1.0	1.48	1.23 (–)
C	2.95	ca.1 (+)	8.34	ca.1 (+)	4.74	1.16 (–)	9.08	1.05 (+)
D	3.62	1.0	6.80	1.08 (–)	3.97	1.21 (–)	2.12	4.02 (–)
E	2.56	ca.1 (+)	4.50	1.15 (+)	3.03	ca.1 (–)	5.12	1.23 (+)
F	1.72	ca.1 (–)	2.61	1.07 (+)	2.73	1.0	3.37	1.11 (+)
G	1.46	ca.1 (+)	1.02	ca.1 (+)	1.34	ca.1 (+)	0.75	ca.1 (+)
H	1.78	1.0	1.21	1.18 (+)	1.39	ca.1 (–)	3.40	1.12 (–)
I	1.55	1.0	1.01	1.28 (–)	1.21	ca.1 (–)	2.61	1.12 (+)
J	2.27	1.0	1.40	1.31 (–)	1.76	1.0	3.86	1.14 (+)
K	1.36	1.0	1.95	1.0	1.79	1.07	1.90	1.0
L	0.19	1.0	0.18	ca.1 (+)	0.23	1.0	0.15	1.0
M	0.58	ca.1 (–)	0.27	ca.1 (–)	0.57	ca.1 (–)	0.47	ca.1 (–)

[a]Column: 25 x 0.20 (i.d.) cm; eluent: *n*-hexane–2-propanol (97/3, v/v); flow rate: 0.2 mL/min. The signs in parentheses represent the Cotton effect signs at 254 nm of the first-eluted enantiomers. [b]Cited from ref. 40.



Surprisingly, the poly-**2b**- and poly-**4b**-based CSPs also separated three chiral metal complexes **H**–**J** into enantiomers, but their elution orders were completely reversed from each other, despite the fact that the poly-**2b** and poly-**4b** possess the same right-handed helical structure assisted by (*R*)-**A** as supported by the almost identical CD spectral patterns with the same Cotton effect signs (ESI Fig. S10 and S12†). Further comparison of the resolution results on the CSPs in Table 2 revealed that the poly-**2b**- and poly-**3b**-based CSPs carrying ester groups but different sequences (-CO_2- and -OCO-, respectively) exhibited a rather complementary resolving ability; for example, poly-**3b** resolved the racemates **C** and **K**, which were not separated on poly-**2b**, while the racemates **H**–**J** were resolved well on poly-**2b**, but not separated at all on poly-**3b**.

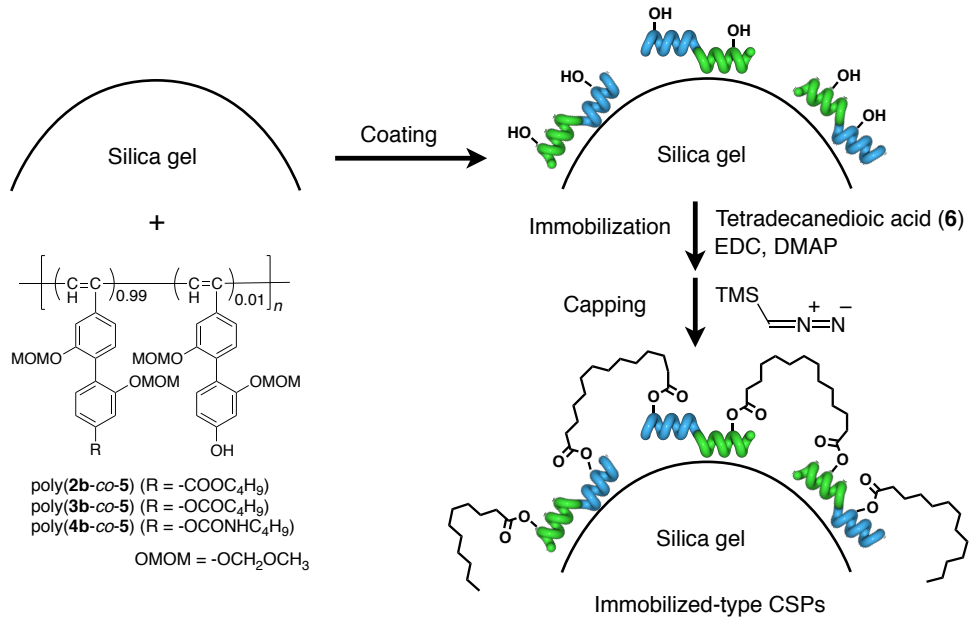
These results suggest the key role of the achiral functional groups introduced at the 4'-position of the biphenyl pendants of the helical PBPAs with a helicity memory during the enantioseparation when used as a CSP. As described above, all the optically active helical poly-**1b**–poly-**4b** used as the coated-type CSPs exhibited characteristic CDs and VCDs with the same Cotton effect signs in the polymer backbone regions and the OMOM groups at the 2,2'-positions of the biphenyl pendants, respectively, when (*R*)-**A** was used as a helix-inducer, clearly indicating that the helical sense of the macromolecular helicity and the twist-sense of the pendant biphenyl axial chirality memorized in the poly-**1b**–poly-**4b** are identical to each other.

Therefore, the observed differences in the chiral recognition abilities between the poly-**1b**–poly-**4b**-based CSPs toward various racemic compounds that include significant differences in their resolving powers and reversals of the elution orders of some particular racemates are ascribed to the difference in the functionalities of the achiral pendant groups introduced on the biphenyl pendants, which may be arranged into a preferred-handed helical array along the helical polymer backbones induced by the macromolecular helicity memory. Thus, the higher enantioselectivities of the poly-**2b** and poly-**4b** mostly arise from the polar ester (-CO_2-) and carbamate residues at the pendants, respectively, which can serve as efficient chiral recognition sites by hydrogen bonding with the racemates.

Chiral recognition abilities of PBPA-based immobilized-type CSPs for HPLC

Unlike the poly-**1a**-based coated-type CSP, showing a unique switchable enantioseparation,[39] poly-**2**–poly-**4** cannot be applied to develop such a switchable coated-type CSP because these polymers are readily soluble in a solution containing (*S*)- or (*R*)-**A** independent of the alkyl chain length. We then developed a facile method to immobilize poly-**2b**–poly-**4b** onto silica gel surface through chemical bonding (cross-linking) between the polymer chains (immobilized-type CSP) according to Scheme 2.

Monomers **2b**–**4b** were first copolymerized with a small amount of (2,2'-bis(methoxymethoxy)-4'-hydroxy-4-biphenylyl)acetylene (**5**) bearing a hydroxy group at the 4'-position of the biphenyl pendant at the feed ratio of [**2b**–**4b**]/[**5**] (99/1, mol/mol) using $[Rh(nbd)Cl]_2$ as a catalyst in THF in the presence of Et_3N at 30 °C, producing the high molecular weight copolymers of poly(**2b**-*co*-**5**), poly(**3b**-*co*-**5**) and poly(**4b**-*co*-**5**) (M_n > 5.3 × 10^5), respectively, in high yields (> 95%) (Table 3). The copolymers also formed an excess one-handed helical structure induced by the interaction with (*R*)-**A** in toluene, thus showing intense ICDs being comparable to those of the corresponding homopolymers (ESI Fig. S33–S35†), suggesting that a small amount of the **5** unit (*ca.* 1 mol%) incorporated into the copolymers did not affect the helicity induction behavior.



Scheme 2 Schematic illustration of immobilization of as-prepared poly(**2b**-*co*-**5**), poly(**3b**-*co*-**5**) and poly(**4b**-*co*-**5**) onto silica gel by cross-linking the copolymers with tetradecanedioic acid (**6**).

Table 3 Copolymerization results of **2b**–**4b** and **5** with $[Rh(nbd)Cl]_2$ in THF/Et_3N at 30 °C for 3 h[a]

Run	M_1 (mol%)	M_2 (mol%)	Sample code	Yield (%)[b]	M_n $\times 10^5$ [c]	M_w/M_n [c]
1	**2b** (99)	**5** (1)	poly(**2b**-*co*-**5**)	96	9.8	1.8
2	**3b** (99)	**5** (1)	poly(**3b**-*co*-**5**)	95	8.5	1.7
3	**4b** (99)	**5** (1)	poly(**4b**-*co*-**5**)	96	5.3	2.1

[a][Monomer] = 0.5 M, $[Rh(nbd)Cl]_2$ = 0.005 M. [b]Methanol insoluble part. [c]Determined by SEC (polystyrene standards) with THF as the eluent.

The immobilizations of the as-prepared poly(**2b**-*co*-**5**), poly(**3b**-*co*-**5**) and poly(**4b**-*co*-**5**) onto silica gel were performed by cross-linking between the hydroxy groups of the copolymer chains coated onto silica gel using a dicarboxylic acid, tetradecanedioic acid (**6**), as a cross-linker promoted by condensing reagents (Scheme 2). During this process, chemical bond formation between the hydroxyl groups of the copolymer chains and the silanol groups of silica surface could not be excluded. The column packing materials composed of the cross-linked PBPAs (Si-poly(**2b**-*co*-**5**), Si-poly(**3b**-*co*-**5**) and Si-poly(**4b**-*co*-**5**)) immobilized onto silica gel were obtained after removing the non-immobilized PBPAs and unreacted/partially reacted **6** by washing with THF, dichloromethane and ethanol (see the Supporting Information (SI)), then packed into stainless-steel columns (25 cm × 0.20 cm (i.d.)) by a conventional high-pressure slurry packing technique.[46,47] The contents of the immobilized polymers onto silica gel were estimated by thermogravimetric (TG) analyses as 9.9 (Si-poly(**2b**-*co*-**5**)), 10 (Si-poly(**3b**-*co*-**5**)) and 9.2 wt% (Si-poly(**4b**-*co*-**5**)) (ESI Table S1†).

The as-prepared Si-poly(**3b**-*co*-**5**)-based HPLC column was filled with a solution of (*R*)-**A** in toluene (50/50, v/v) and allowed to stand at rt for 24 h to induce a preferred-handed (right-handed) helical conformation in the immobilized polymer backbone. The toluene solution of (*R*)-**A** in the column was then completely removed by passing a *n*-hexane–2-propanol (97/3, v/v) mixture through the column. During this process, the induced right-handed (*P*) helical conformation of the immobilized poly(**3b**-*co*-**5**) could be memorized, resulting in the CSP that consisted of *P*-Si-poly(**3b**-*co*-**5**) with a right-handed helicity memory (Fig. 3Aa). The chromatogram for the resolution of the 33% enantiomeric excess (ee) ((–)-isomer rich) of **D** on the immobilized-type *P*-Si-poly(**3b**-*co*-**5**) column is shown in Fig. 3Ba. The major (–)-enantiomer eluted first followed by the (+)-enantiomer, and they were almost completely separated with an α value of 1.12, which is slightly lower than that on the corresponding *P*-poly-**3b**-based coated-type CSP (α = 1.21) with the same right-handed helicity memory. This result indicated that the noncovalent helicity induction and subsequent memory of the induced helicity method can be applied to PBPA derivatives immobilized onto silica gel. The relatively lower enantioselectivity of the immobilized-type *P*-Si-poly(**3b**-*co*-**5**) may arise from the cross-linked structure of poly(**3b**-*co*-**5**), to which a fully one-handed helical conformation may not be completely induced.

Prior to inverting the helical sense of the *P*-Si-poly(**3b**-*co*-**5**), the column was filled with dichloromethane, a good solvent for

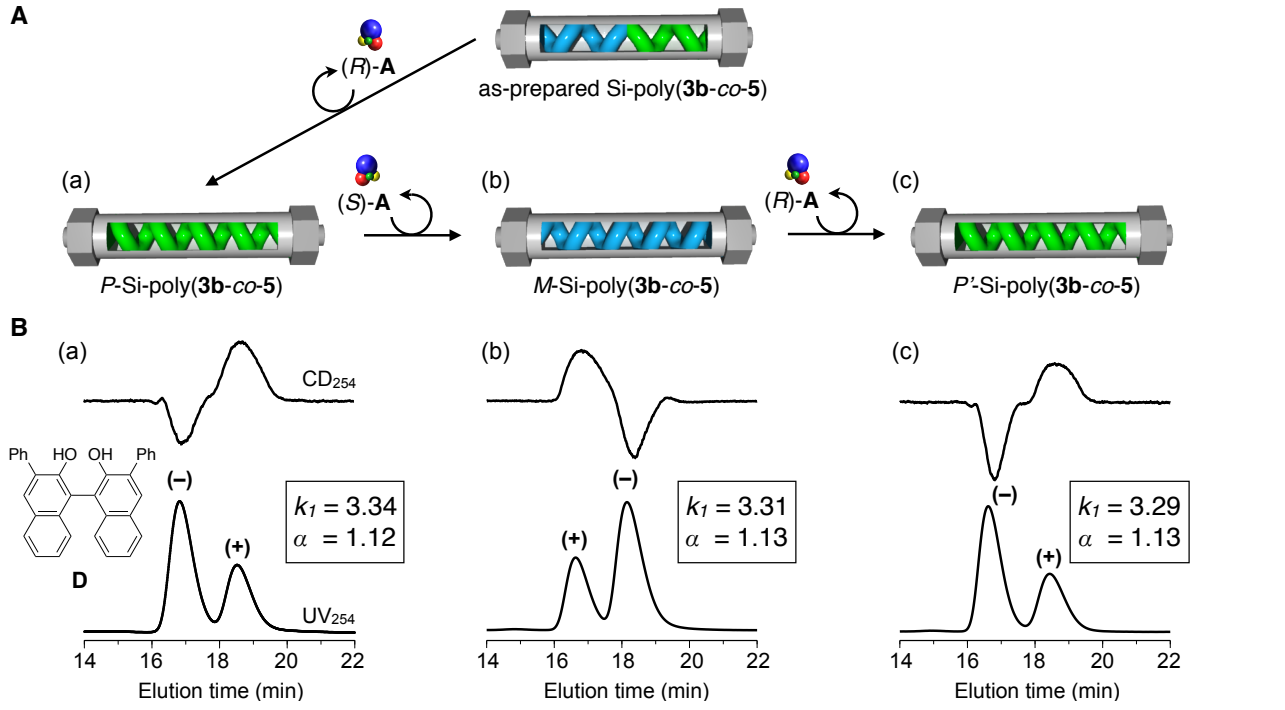


Fig. 3 (A) Schematic illustration of a switchable CSP for HPLC separation of enantiomers based on reversible switching and memory of the macromolecular helicity of Si-poly(**3b**-*co*-**5**) by sequential treatment with (*R*)-, (*S*)- and (*R*)-**A** in an alternating manner, thus producing right-handed *P*-Si-poly(**3b**-*co*-**5**) (a), left-handed *M*-Si-poly(**3b**-*co*-**5**) (b) and right-handed *P*′-Si-poly(**3b**-*co*-**5**) (c). (B) Chromatograms for the resolution of 33% ee of **D** ((–)-isomer rich) on *P*-Si-poly(**3b**-*co*-**5**) (a), *M*-Si-poly(**3b**-*co*-**5**) (b) and *P*′-Si-poly(**3b**-*co*-**5**) (c) prepared by treatment with (*R*)-, (*S*)- and (*R*)-**A**, respectively, at ca. 10 °C.

poly(**3b**-*co*-**5**), which allowed the right-handed helix memory of the *P*-Si-poly(**3b**-*co*-**5**) to erase at 25 °C within 2 h. The column was then treated with a solution of (*S*)-**A** in toluene (50/50, v/v) followed by rinse with a *n*-hexane–2-propanol mixture (97/3, v/v) to completely remove (*S*)-**A**, thus producing the *M*-Si-poly(**3b**-*co*-**5**)-based CSP with an opposite left-handed helicity memory (Fig. 3Ab), which also resolved the enantiomers of **D** with a completely reversed elution order; the minor (+)-enantiomer eluted first, then the major (–)-enantiomer with almost the similar k_1 and α values (Fig. 3Bb). Further treatment of the *M*-Si-poly(**3b**-*co*-**5**) with (*R*)-**A** in toluene (50/50, v/v) in the column followed by passing a *n*-hexane–2-propanol (97/3, v/v) mixture through the column regenerated the CSP (*P*'-Si-poly(**3b**-*co*-**5**)) with the right-handed helicity memory (Fig. 3Ac). As anticipated, the *P*'-Si-poly(**3b**-*co*-**5**) column separated the enantiomers of **D** (Fig. 3Bc) with virtually the same enantioselectivity and elution order as those on the *P*-Si-poly(**3b**-*co*-**5**) column (Fig. 3Ba). As a result, the elution orders of the enantiomers of **D** as well as **C** during chiral chromatography were repeatedly switched using the Si-poly(**3b**-*co*-**5**)-based immobilized-type CSP based on the reversible switching of the helical sense of the Si-poly(**3b**-*co*-**5**) followed by memory of the macromolecular helicity, while maintaining their chiral recognition abilities (Fig. 3 and ESI Table S3†).

Similar switchable enantioseparations assisted by reversible switching of the macromolecular helicity memory were also possible for the Si-poly(**2b**-*co*-**5**)- and Si-poly(**4b**-*co*-**5**)-based immobilized-type columns by sequential treatment with (*R*)- and (*S*)-**A** in an alternate manner, which resolved some selected enantiomers **E**, **I**, **J** and **D**, **E**, **H**, respectively, whose elution orders were definitely switched in series (ESI Table S2 and S4†).

Conclusions

In summary, we have synthesized a series of PBPA derivatives (poly-**1**–poly-**4**) bearing different types of polar functional groups, such as ether, ester, acyloxy and carbamate groups, at the 4'-position of the biphenyl pendants. The macromolecular helicity of the polymer backbones together with the axial chirality of the biphenyl pendants could be induced in these polymers through noncovalent chiral interactions with optically active alcohols and further almost completely memorized after complete removal of the chiral inducers regardless of the kind of polar functional groups of the biphenyl pendants. The stabilities of the helicity memory of the PBPAs significantly increased by introducing the polar functional groups at the pendants when compared to that of PBPAs bearing ether groups, probably due to cooperative intramolecular interactions between the neighboring polar functional groups. The helicity-memorized PBPAs with ester or carbamate groups were found to show characteristic chiral recognition abilities depending on the functionalities of the achiral 4'-pendant groups when applied to the coated-type CSPs for HPLC, indicating that the polar groups of the biphenyl pendants play a key role as chiral recognition sites. We developed a facile method to immobilize PBPA-based copolymers onto silica gel surface by cross-linking between the polymer chains. By applying the helicity induction and its memory strategy, we successfully produced a series of immobilized-type CSPs capable of switching the helical sense of the immobilized PBPAs with the macromolecular helicity memory in the columns by sequential treatment with (*R*)- and (*S*)-alcohols in an alternate manner, resulting in the switchable CSPs through which the elution order of enantiomers can be controlled and repeatedly switched. We believe that practically useful elution order switchable PBPA-based CSPs showing a higher enantioselectivity will be developed by further modification of the pendant group functionalities.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

1. G. Blaschke, H. P. Kraft, K. Fickentscher and F. Kohler, *Arzneimittelforschung*, 1979, **29-2**, 1640-1642.
2. E. J. Ariens, *Med. Res. Rev.*, 1986, **6**, 451-466.
3. B. Waldeck, *Chirality*, 1993, **5**, 350-355.
4. A. G. Rauws and K. Groen, *Chirality*, 1994, **6**, 72-75.
5. N. M. Maier, P. Franco and W. Lindner, *J. Chromatogr. A*, 2001, **906**, 3-33.
6. S. Andersson and S. G. Allenmark, *J. Biochem. Biophys. Methods*, 2002, **54**, 11-23.
7. B. Kasprzyk-Hordern, *Chem. Soc. Rev.*, 2010, **39**, 4466-4503.
8. W. H. Brooks, W. C. Guida and K. G. Daniel, *Curr. Top. Med. Chem.*, 2011, **11**, 760-770.
9. Q. Zhou, L. S. Yu and S. Zeng, *Drug Metab. Rev.*, 2014, **46**, 283-290.
10. C. J. Welch, *J. Chromatogr. A*, 1994, **666**, 3-26.
11. S. Fanali, *J. Chromatogr. A*, 2000, **875**, 89-122.
12. E. R. Francotte, *J. Chromatogr. A*, 2001, **906**, 379-397.
13. F. Gasparrini, D. Misiti and C. Villani, *J. Chromatogr. A*, 2001, **906**, 35-50.
14. T. J. Ward and A. B. Farris, *J. Chromatogr. A*, 2001, **906**, 73-89.
15. T. Nakano, *J. Chromatogr. A*, 2001, **906**, 205-225.
16. R. J. Steffeck, Y. Zelechonok and K. H. Gahm, *J. Chromatogr. A*, 2002, **947**, 301-305.
17. V. A. Davankov, *J. Chromatogr. A*, 2003, **1000**, 891-915.
18. I. D'Acquarica, F. Gasparrini, D. Misiti, M. Pierini and C. Villani, in *Advances in Chromatography, Vol 46*, eds. E. Grushka and N. Grinberg, 2008, vol. 46, pp. 109-173.

19. M. Laemmerhofer and W. Lindner, in *Advances in Chromatography, Vol 46*, eds. E. Grushka and N. Grinberg, 2008, vol. 46, pp. 1-107.
20. Y. Okamoto, K. Suzuki, K. Ohta, K. Hatada and H. Yuki, *J. Am. Chem. Soc.*, 1979, **101**, 4763-4765.
21. H. Yuki, Y. Okamoto and I. Okamoto, *J. Am. Chem. Soc.*, 1980, **102**, 6356-6358.
22. Y. Okamoto, S. Honda, I. Okamoto, H. Yuki, S. Murata, R. Noyori and H. Takaya, *J. Am. Chem. Soc.*, 1981, **103**, 6971-6973.
23. Y. Okamoto and T. Nakano, *Chem. Rev.*, 1994, **94**, 349-372.
24. W. H. Pirkle and T. C. Pochapsky, *Chem. Rev.*, 1989, **89**, 347-362.
25. Y. Okamoto and E. Yashima, *Angew. Chem. Int. Ed. Engl.*, 1998, **37**, 1020-1043.
26. E. Yashima, *J. Chromatogr. A*, 2001, **906**, 105-125.
27. C. Yamamoto and Y. Okamoto, *Bull. Chem. Soc. Jpn.*, 2004, **77**, 227-257.
28. Y. Okamoto and T. Ikai, *Chem. Soc. Rev.*, 2008, **37**, 2593-2608.
29. E. Yashima, K. Maeda, H. Iida, Y. Furusho and K. Nagai, *Chem. Rev.*, 2009, **109**, 6102-6211.
30. A. Aranyi, I. Ilisz, N. Grecso, R. Csuetoertoeki, I. Szatmari, F. Filloep and A. Peter, *J. Pharm. Biomed. Anal.*, 2013, **76**, 183-191.
31. J. Shen and Y. Okamoto, *Chem. Rev.*, 2016, **116**, 1094-1138.
32. E. Yashima, N. Ousaka, D. Taura, K. Shimomura, T. Ikai and K. Maeda, *Chem. Rev.*, 2016, **116**, 13752-13990.
33. B. Yao, F. Zhan, G. Yu, Z. Chen, W. Fan, X. Zeng, Q. Zeng and W. Weng, *J. Chromatogr. A*, 2009, **1216**, 5429-5435.
34. L. Chankvetadze, N. Ghibradze, M. Karchkhadze, L. Peng, T. Farkas and B. Chankvetadze, *J. Chromatogr. A*, 2011, **1218**, 6554-6560.
35. L. Mosiashvili, L. Chankvetadze, T. Farkas and B. Chankvetadze, *J. Chromatogr. A*, 2013, **1317**, 167-174.
36. M. Gegenava, L. Chankvetadze, T. Farkas and B. Chankvetadze, *J. Sep. Sci.*, 2014, **37**, 1083-1088.
37. J. A. Perry, J. D. Rateike and T. J. Szczerba, *J. Chromatogr.*, 1987, **389**, 57-64.
38. M. Okamoto, *J. Pharm. Biomed. Anal.*, 2002, **27**, 401-407.
39. K. Shimomura, T. Ikai, S. Kanoh, E. Yashima and K. Maeda, *Nat. Chem.*, 2014, **6**, 429-434.
40. R. Ishidate, K. Shimomura, T. Ikai, S. Kanoh and K. Maeda, *Chem. Lett.*, 2015, **44**, 946-948.
41. R. Ishidate, T. Ikai, S. Kanoh, E. Yashima and K. Maeda, *Chirality*, 2017, **29**, 120-129.
42. T. Ikai and Y. Okamoto, *Chem. Rev.*, 2009, **109**, 6077-6101.
43. Y. Kishimoto, P. Eckerle, T. Miyatake, T. Ikariya and R. Noyori, *J. Am. Chem. Soc.*, 1994, **116**, 12131-12132.
44. C. I. Simionescu, V. Percec and S. Dumitrescu, *J. Polym. Sci., Part A: Polym. Chem.*, 1977, **15**, 2497-2509.
45. Y. Kishimoto, P. Eckerle, T. Miyatake, M. Kainosho, A. Ono, T. Ikariya and R. Noyori, *J. Am. Chem. Soc.*, 1999, **121**, 12035-12044.
46. Y. Okamoto, R. Aburatani and K. Hatada, *J. Chromatogr.*, 1987, **389**, 95-102.
47. Y. Okamoto, M. Kawashima and K. Hatada, *J. Chromatogr.*, 1986, **363**, 173-186.
48. The racemic **A** could not be separated at all on these PCBA-based CSPs (α = 1.0), despite the fact that the main chain helicity and pendant axial chirality of these PCBAs were efficiently induced through non-covalent interaction with optically active **A**.