

Resolution of Enantiomers Using Sugar-Carrying Polyisocyanides as Chiral Stationary Phases for HPLC

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3,5-Dimethylphenylcarbamate derivatives of α-/β-glucose and α-/β-galactose-carrying helical poly(phenyl isocyanide)s were used as chiral stationary phases (CSPs) for HPLC to estimate their chiral recognition abilities. CD spectroscopy suggested that the helix sense in these rigid helical polymers may be regulated by the chirality of the α- or β-anomeric center of the sugar moieties. Some 10 different types of racemates with functional groups were completely or partially resolved depending on the stereostructure of the pendant sugars. The enhanced chiral recognition ability is attributable to the three-dimensionally regulated sugar arrays along the helical backbone; that is, the CSP of the α-glucose-carrying helical poly(phenyl isocyanide) exhibited more effective enantioseparation than that of the corresponding flexible poly(*N*-phenylacrylamide). The CSPs of the galactose-carrying poly(phenyl isocyanide)s showed the resolving ability for broader racemates than those of the glucose-type poly(phenyl isocyanide)s. Especially, the CSP of the α-galactose-carrying poly(phenyl isocyanide) separated the largest number of racemates.

Saccharide chains on cell surfaces play important roles in cell–cell recognition as well as in infection by viruses and bacteria.[1] Synthetic polymers carrying various kinds of pendant saccharide chains are useful tools to investigate saccharide-recognition events.[2] The clustered saccharide chains along flexible polystyrene and polyacrylamide backbones were reported to strongly bind to a variety of carbohydrate-binding proteins (lectins).[3] However, glycosylated poly(phenyl isocyanide)s exhibited few specific interactions with lectins, in spite of the multivalent saccharide arrays.[4] The saccharide arrays of the glycosylated poly(phenyl isocyanide)s were regulated in a helical manner so tightly that they could neither access nor induce-fit to the binding sites of the lectins. A polymer with a rigid backbone, such as polyisocyanide, may be suitable for the recognition of small compounds.

The phenylcarbamate derivatives of naturally occurring chiral polymers, cellulose and amylose, have been commercialized as chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC) to separate many racemates including drugs.[5] The chiral separation abilities of these polysaccharide derivatives originate from the crowded polar phenylcarbamate residues along the highly stereoregular polymer backbones. CSPs consisting of synthetic polymers, such as polyamides, polyacrylamides, and one-handed helical polymethacrylates, have been extensively studied.[6] Although polyisocyanides are also non-natural helical polymers, few papers have reported on their chiral recognition ability.[7]

In this respect, it is of interest to investigate the chiral recognition ability of glycosylated poly(phenyl isocyanide)s carrying the chirality of both the saccharide side chain and polyisocyanide main chain. We prepared the phenylcarbamates of the glucose- and galactose-carrying polyisocyanides (**1**–**4**) (Fig. 1) and evaluated their chiral recognition abilities as CSPs for HPLC, in comparison with that of the flexible backbone of poly(*N*-phenylacrylamide) (**5**).

Materials and Methods

Measurements. The ^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini-200 (200 MHz for ^{1}H) and VXR-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) NMR spectrometers. The chemical shifts are reported in ppm (δ) relative to Me_4Si or residual non-deuterated solvent. The IR spectra were recorded on a JASCO FT/IR-230 Fourier-transform infrared spectrometer. Circular dichroism (CD) spectroscopy was measured by a JASCO J-720L spectropolarimeter using a 10 mm quartz cell.

Materials. Macroporous spherical silica gel (Daiso gel SP-1000) with a mean particle size of 7 μm and a mean pore diameter of 100 nm was supplied by Daiso Chemical (Osaka, Japan). Analytical-grade solvents were carefully dried and distilled before preparing the CSPs.

Synthesis of Poly(phenyl isocyanide)s Carrying 3,5-Dimethylphenyl-carbamoylated Sugars. The synthesis of the glycosylated poly(phenyl isocyanide)s was carried out by the previously described method,[4] as shown in Scheme 1, starting from *p*-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside, as an example. Briefly, the hydrogenation of the nitro group to the amine, followed by formylation with acetic formic anhydride in ethyl formate, gave the *N*-formyl-amino group, which was then converted to an isocyano group by phosphoryl chloride and triethylamine. The isocyano derivative was polymerized with nickel(II) chloride in a mixture of chloroform

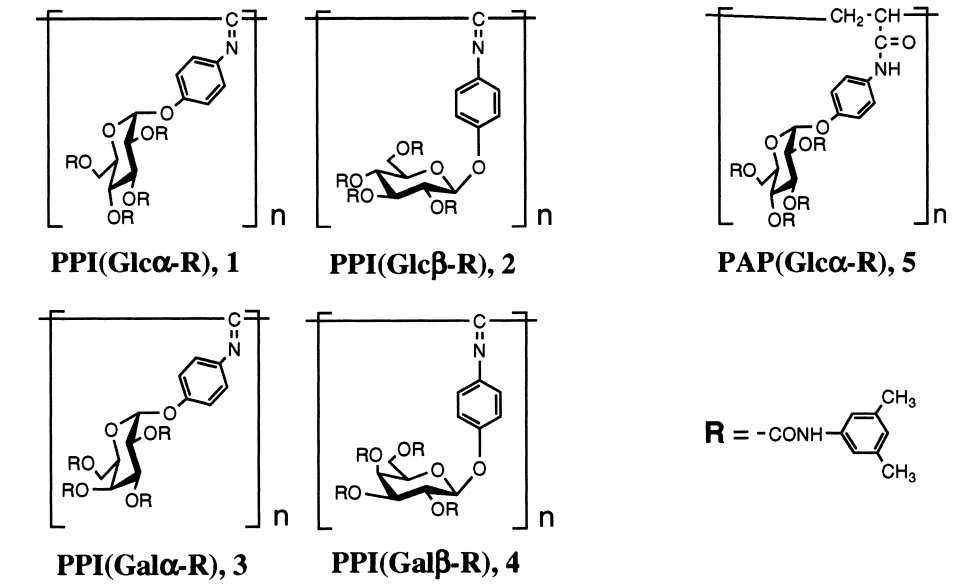
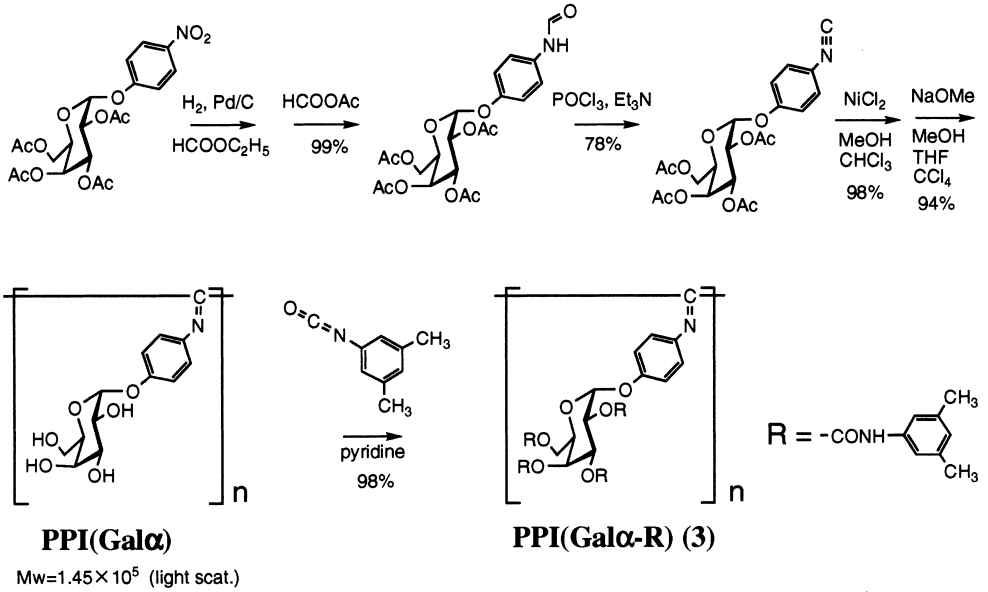


Fig. 1. Rigid helical poly(phenyl isocyanide)s carrying phenylcarbamoylated sugars (**1**–**4**), together with the corresponding flexible analogue poly(*N*-phenylacrylamide) polymer (**5**) as a control.



Scheme 1. Synthesis of rigid helical poly(phenyl isocyanide) carrying monosaccharide.

and methanol.[8] The polymer was deprotected with a catalytic amount of sodium methoxide in a mixture of methanol, tetrahydrofuran, and tetrachloromethane. Complete deacetylation of the polymer was carried out by the addition of water to the reaction solution.

Carbamoylation was performed by the conventional procedure.[9] The α-glucose-carrying poly(phenyl isocyanide) (0.39 g, 1.39 mmol) was dispersed in dry pyridine (8 mL), and an excess amount of 3,5-dimethylphenyl isocyanate (1.22 mL, 8.3 mmol) was added to the suspended solution. The mixture was then stirred at 80 °C for 72 h. The resulting phenylcarbamoylated product was isolated as a methanol–water (5:1, v/v) insoluble part (1.20 g, yield 98%). ^{1}H NMR (500 MHz, Me_2SO-d_6) δ 4.1–4.9 (br, m, other protons from sugar), 5.1 (br, s, H-1), 9.3 (br, s, –OCO*NH*–). IR (KBr) 3317, 3301, 1722, 1616, and 1552 cm^{-1}.

Preparation of Chiral Columns.[9] Each poly(phenyl isocyanide) derivative (0.75 g) was dissolved in THF (15 mL) to coat the silica gel. Macroporous silica gel was treated with a large excess of (3-aminopropyl)triethoxysilane in dry benzene in the presence of a catalytic amount of dry pyridine at 80 °C overnight. The silanized silica gel (3.0 g) was wetted with one portion of the polymer solution as uniformly as possible; the solvent was then evaporated under reduced pressure. The wetting/drying procedure was repeated several times to coat the silica gel with the polymer. The resulting silica gel was packed into a stainless-steel tube (25 cm × 0.46 (i.d.) cm) by a conventional high-pressure slurry-packing technique using a Mod-

el CCP-085 Econo packer pump (Chemco, Osaka, Japan). 1,3,5-Tri-*t*-butylbenzene was used as a non-retained compound to estimate the dead time (t_0).

Apparatus. Chromatographic experiments were performed on a JASCO PU-980 chromatograph equipped with a UV (JASCO UV-970) and a polarimetric (JASCO OR-990) detector at room temperature. A solution of a racemate (1–10 μL) was injected into the chromatographic system with a Rheodyne Model 7125 injector. A hexane-2-propanol (98/2) mixture was used as the eluent at a flow rate of 0.5 mL/min, unless otherwise specified.

Results and Discussion

Circular Dichroism (CD) Spectra. The CD spectra of the 3,5-dimethylphenylcarbamates in THF are compared in Fig. 2. The α-glycoside polymers **1** and **3** showed a negative Cotton effect, whereas the β-glycoside polymers **2** and **4** showed a positive Cotton effect for the absorption due to phenyl isocyanide residues above 250 nm. Since the negative and positive Cotton effects of polyisocyanides are respectively assignable to right-handed and left-handed helices,[10] the helix sense in these polymers may be regulated by the chirality of the α- or β-anomeric center. Each intensity of the CD spectra was smaller than those expected for the completely right- or left-handed helices, indicating that these poly(phenyl isocyanide)s are a mixture of the right- and left-handed helical polymers or sequences.

The helix senses of the present phenylcarbamoylated polymers were the same as those of the acetylated precursors,[4] although the intensities [θ] of the present polymers became slightly smaller than those of the precursors. Based on molecular dynamics calculations, we assumed that the helices of the polyisocyanide-bearing acetylated and nonprotected saccharide moieties become more slender and more stretched than the one estimated from the 4_1 helices reported for the other polyisocyanides.[11] This is probably due to steric repulsion among the highly crowded, bulky saccharide chains. In the same way, the more bulky phenylcarbamoyl substituents may make a helical structure of these polymers more slender. In addition, it is also speculated that the number of helical reversals in a polymer chain may be increased; that is, the length of the one-handed helical segment in the polymer would become shorter. Green et al.[12] pointed out that in a statistical thermodynamic study of polyisocyanates, in principle, dynamic helix reversals in the polymer chain can randomly occur in the backbone of the polymer chain. The existence of such backbone motions can also be postulated for the present polyisocyanide.

Chromatographic Enatioseparation. Figure 3 shows a chromatogram of the resolution of racemic 1,2,2,2-tetraphenylethanol (**6**) on CSP-**1** (**1**, PPI(Glcα-R)). Enantiomers elute at retention times of t_1 and t_2. The capacity factors, k_1' [$= (t_1 - t_0)/t_0$] and k_2' [$= (t_2 - t_0)/t_0$], were 0.39 and 0.62, respectively. The separation factor, α [$= k_2'/k_1'$], and the resolution factor, R_s [$= 2(t_2 - t_1)/(w_1 + w_2)$], were found to be 1.59 and 1.83, respectively. The α value expresses the chiral recognition ability of a CSP, and the R_s value depends on both the α value and the sharpness of the peaks. The band widths of the first- and second-eluted enantiomers on the base line are shown as w_1 and w_2. R_s can be determined when the two peaks are almost completely separated. The chromatogram in Fig. 3 shows the expeditious complete separation of enantiomers with rather sharp peaks. Table 1 summarizes the results of the

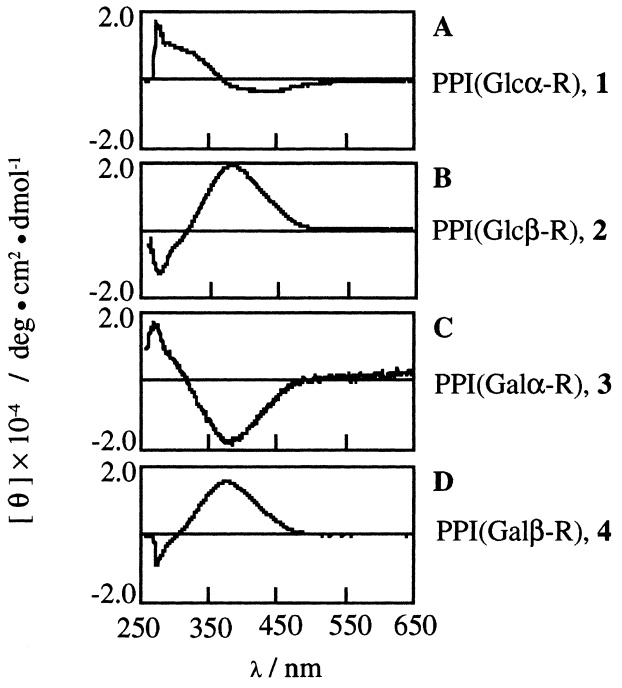


Fig. 2. CD spectra of 3,5-dimethylphenylcarbamoylated glycopolyisocyanides in THF.

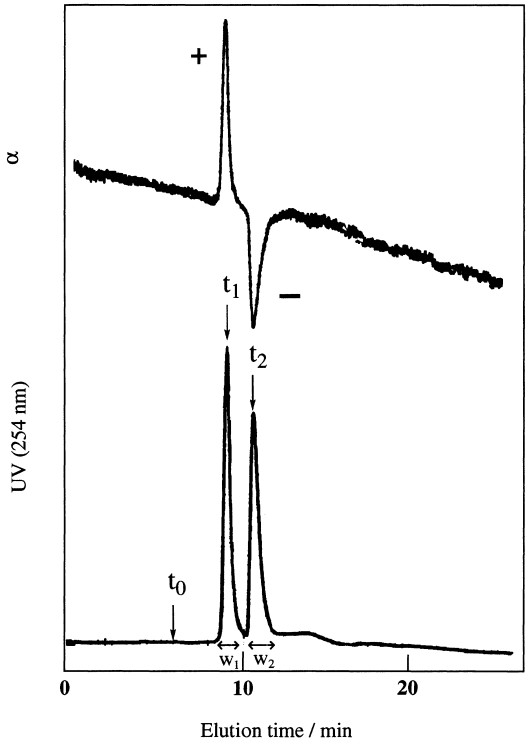


Fig. 3. Separation of 1,2,2,2-tetraphenylethanol (**6**) on CSP-**1**. Eluent: hexane/2-propanol (98/2); flow rate: 0.5 mL/min.

Table 1. Resolution on CSP of 3,5-Dimethylphenylcarbamate Derivatives of Rigid Poly(phenyl isocyanide)s (**1**–**4**) and of Flexible Poly(*N*-phenylacrylamide) (**5**)[a)]

	PPI(Glcα-R), **1**			PPI(Glcβ-R), **2**			PPI(Galα-R), **3**			PPI(Galβ-R), **4**			PAP(Glcα-R), **5**		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
6	0.39 (+)	1.59	1.83	0.49	1.00		0.30 (−)	~1		0.54 (−)	~1		0.73 (+)	1.14	0.72
7	0.80 (−)	1.15	1.16	1.62 (+)	1.24	1.29	1.59 (+)	~1		1.95 (+)	1.07		2.65 (−)	~1	
8	0.38	1.00		0.90 (+)	~1		0.73 (+)	~1		1.02 (+)	~1		0.92	~1	
9	1.56	1.00		2.92 (−)	1.15		2.43 (+)	1.09		2.77 (−)	1.08		1.22	1.00	
10	0.12 (−)	~1		0.21 (−)	~1		0.17 (+)	~1		0.24 (+)	1.16		0.26 (−)	~1	
11	0.55	1.00		1.11 (−)	~1		0.71 (+)	1.34	1.14	1.05 (+)	1.10		1.07	~1	
12	0.95	~1		4.47[b)]	1.00		1.41 (+)	1.37	0.76	3.50 (+)	1.26		0.88	~1	
13	0.29 (+)	~1		0.71 (−)	~1		0.57 (−)	1.07		0.82 (−)	~1		0.79 (+)	~1	
14	0.29 (−)	~1		0.48 (−)	1.13		0.61 (+)	1.47	1.41	0.70	~1		0.52 (+)	~1	
15	2.09	1.00		7.83[b)]	1.00		9.19 (+)	1.02		4.03	1.02		2.17 (+)	1.22	0.77

a) Eluent: hexane/2-propanol (98/2), flow-rate: 0.5 mL/min. The signs in parentheses represent the optical rotation of the first-eluting enantiomers. b) Flow-rate: 1.0 mL/min.

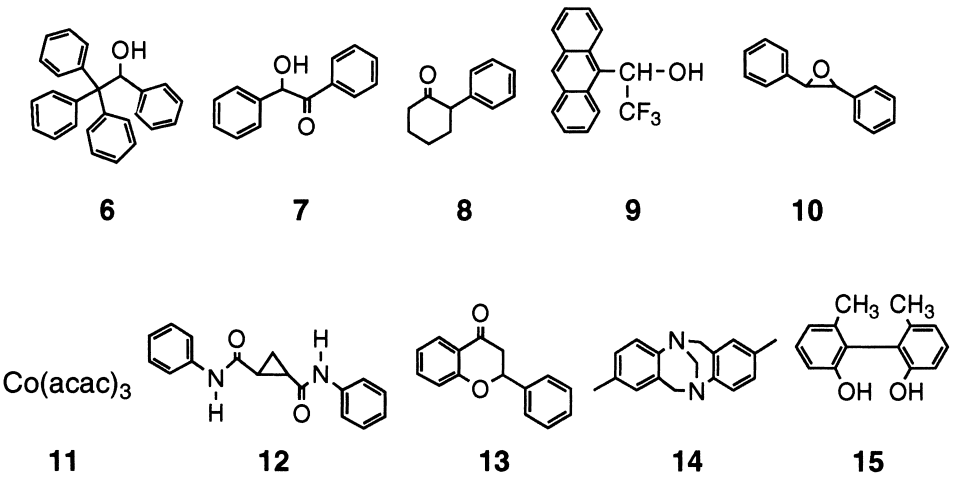


Fig. 4. Structures of racemates **6**–**15**.

enantioseparation of the following 10 racemates with functional groups (Fig. 4): **6**, benzoin (**7**), 2-phenylcyclohexanone (**8**), 1-(9-anthryl)-2,2,2-trifluoroethanol (**9**), (±)-*trans*-stilbene oxide (**10**), tris(acetylacetonate) cobalt(III) (**11**), *trans*-cyclopropane-1,2-dicarboxanilide (**12**), flavanone (**13**), Tröger's base (**14**), and 2,2′-dihydroxy-6,6′-dimethyl-1,1′-biphenyl (**15**). The results were evaluated by the separation factor α.

The effects of the helical backbone on the enantioseparation can be clearly demonstrated by comparing CSP-**1** and CSP-**5**, which have the same α-glucopyranoside structures along the helical and flexible backbones, respectively. In the resolution of racemate **6**, **7**, and **15** on CSP-**1** and CSP-**5**, there existed a distinct difference in the separation factors (α). The helical CSP-**1** almost completely separated racemates **7** and **6** with α separation factors of 1.15 and 1.59, respectively. In contrast, the non-helical CSP-**5** only partially separated racemate **6** and **15** with α values of 1.14 and 1.22. The chiral recognition ability of CSP-**1** is clearly different from that of CSP-**5**, which may be attributable to the helical saccharide arrays along the regulated poly(phenyl isocyanide) backbone.

The chiral recognition ability is dependent on the stereochemistry (C1 and C4) of the sugar moieties in the poly(phenyl isocyanide)s (**1**–**4**). The glucose-type CSPs-**1** and -**2** separated some racemates with larger α and R_s; especially, the α and R_s for racemate **6** on CSP-**1** were the highest among the combinations listed in Table 1. In contrast, the galactose-type CSP-**3** and CSP-**4** showed chiral recognition ability for a broader range of compounds than the glucose-type CSP-**1** and CSP-**2**; that is, six racemates were completely or partially resolved on both CSP-**3** and CSP-**4**. Especially, CSP-**3** separated the largest number of racemates.

The chiral recognition ability also depended on the anomeric configuration (α/β) of the sugar moieties; in other words, the helical structure of the poly(phenyl isocyanide)s induced by various sugars. By comparing the most efficient separations on each CSP, it is found that the α-glycoside-type CSPs have higher enantioseparation abilities than the β-glycoside-type CSPs; the α value (1.59) for **6** on CSP-**1** and those for **11** (1.34), **12** (1.37), and **14** (1.47) on CSP-**3** were larger than those for the corresponding racemates on CSP-**2** and CSP-**4**. Additionally, it was found that the elution order of some enantiomers was reversed on these CSPs. For example, (−)-**9** eluted first on CSP-**2** and CSP-**4**, but second on CSP-**3**, and (−)-**14** eluted first on CSP-**2**, and second on CSP-**3**. Also, (−)-**7** eluted first on CSP-**1** and (+)-**7** on CSP-**2**. On the other hand, the selectivities of enantiomers on CSP-**1** and CSP-**5** were similar.

Therefore, the selectivity of enantiomers on poly(phenyl isocyanides) seems to be mainly dependent on the conformation and the anomeric configuration of the tethered monosaccharide derivative.

The chiral discrimination mechanism of these glycopoly(phenyl isocyanide)s is still obscure. The discrimination may be ascribed to the different chiral environment constructed by the different sugar units. The phenylcarbamoylated monosaccharide residues arranged along the main chain may induce a prevailing one-handed helical structure, which can contribute to enantioseparation.

The separation factors (α) on CSP-**1** were smaller than those on other CSPs for various racemates, except for **6**. This may be related to the lower one-handed helical structure of PPI(Glcα-R) (**1**) than **2**, **3**, and **4**, as suggested by the CD measurement of the CSPs. The smaller CD intensity [θ] of **1** compared with the others suggests that the obtained enantioseparation data for various racemates may not show the inherent chiral recognition ability of CSP-**1**. The present α-glucose carrying polyisocyanide has helix reversals in the polymer backbone, and the length of the one-sided helical segment in polymer seems to be short. If the more regulated PPI(Glcα-R) (**1**) with the one-handed helical sequence could be prepared, the CSP-**1** might perform excellent enantioseparations. The future direction of this study will be an examination of appropriate conditions for the more stereo-regulated polymerization and the suppression of dynamic helix reversal in a polymer.

In conclusion, the CSPs of the 3,5-dimethylphenylcarbamoyled glycosyl poly(phenyl isocyanide)s effectively separated some enantiomers, depending on the saccharide structures. The highly crowded saccharide arrays along the rigid helical poly(phenyl isocyanide) backbones may play an important role for chiral recognition. Their chiral separation ability would be significantly enhanced if the one-handed helical poly(phenyl isocyanide)s could be prepared.

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