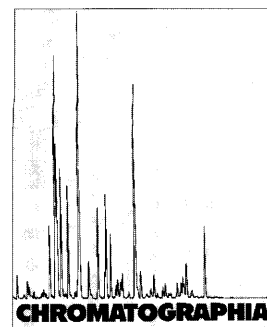


Selectivity Enhancement of Diastereomer Separation in RPLC Using Crystalline-Organic Phase-Bonded Silica



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Key Words

Column liquid chromatography
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Selectivity enhancement
Carbonyl- π interaction
Phase transition

Summary

Poly(octadecyl acrylate) exists in a crystalline state at room temperature. This crystalline state was formed even after being grafted onto silica through the terminal group of the polymer. When this polymer-grafted silica (Sil-ODA_n) was used as a reversed phase liquid chromatography packing material, better selectivity for diastereomerized phenylethylamines derivatized by (S)-(-)-(2,3-naphthalenedicarboximidyl)propionyl fluoride was observed at room temperature than was observed for simple octadecylated silica (ODS). On the other hand, increasing temperature reduced the selectivity to be close to that of ODS. Additional experiments showed the better selectivity derived from Sil-ODA_n was due to both the highly-oriented structure of the polymer and the carbonyl- π interaction with the diastereomer. A theoretical investigation of this carbonyl- π interaction is also described briefly.

Introduction

Diastereomerization of enantiomeric isomers is effective for chiral separation of the isomers. When this technique is combined with reversed-phase liquid chromatography (RPLC), it yields a very convenient method of analysis of enantiomer mixtures. Therefore many diastereomerizing reagents have been developed [1–7]: *o*-phthalaldehyde-*N*-acetyl-L-cystein [1], (+)-1-(9-fluorenyl)ethylchloroformate [2], (a2)-2'-methoxy-1,1'-binaphthyl-2-carboxylate [3], (S)-(-)-(2,3-naphthalenedicarboximidyl)-

propionyl fluoride [4] and chiral monohalo-triazines [5] for racemic amines and/or amino acids, and S(+)-1-methyl-2-(2,3-naphthalimido)ethyl trifluoro-methanesulfonate [6] for racemic carboxylic acids.

These diastereomerizing reagents can be characterized by the fact that chromophoric groups are included for sensitive detection and also because chiral separations are carried out by utilizing the hydrophobicity (or polarity) differences between the resulting diastereomers. Therefore, simple hydrophobized silica such as octadecylated silica (ODS) has been used

mainly as an organic stationary phase in RPLC. However, since the hydrophobicity difference between diastereomers is rather small, ODS usually shows low selectivity. Therefore, we focus on the fact that the above-mentioned diastereomerizing reagents have chromophoric groups which can work as a π - π interaction source. From these considerations, we applied poly(long-chain alkyl acrylate)-grafted silicas (Sil-ODA_n and Sil-DCA_n) as packing materials for diastereomer separation in an RPLC mode. These polymeric organic phases have been developed by us [8–10] and are very attractive for the following reasons: (1) they have many carbonyl groups that act as π -electron sources and (2) they undergo a temperature-induced crystalline-to-isotropic phase transition as schematically illustrated in Figure 1. In this paper, we wish to report that diastereomeric separation can be enhanced through high orientation of the carbonyl groups on silica.

Experimental

Stationary Phases

Poly(octadecyl acrylate) with terminal reactive groups, ODA_n, was prepared by the method reported previously [9, 10]. The average degree of polymerization (*n*) of the polymer was determined by ¹H-NMR spectroscopy to be 24. ODA₂₄ is readily grafted onto porous silica by mixing in tetrachloromethane at reflux temperature. Fuji Silysia Super Micro Bead silica gel (100A-5D, diameter 5 μ m, pore

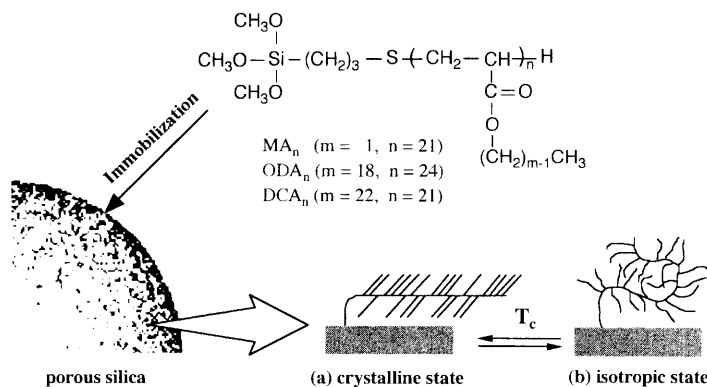


Figure 1. Chemical structures of polymers (ODA_n, DCA_n and MA_n) as immobilized organic phases and schematic illustration of crystalline-to-isotropic phase transition of ODA_n and DCA_n on silica.

size 146 Å, specific surface area 330 cm² g⁻¹) was used as the porous silica. The amount of ODA₂₄ immobilized was determined by elemental analysis to be 18.0 wt%. Poly(docosyl acrylate)-immobilized silica (Sil-DCA_n) was prepared using the same procedure. Docosyl acrylate of mp. 47 °C was synthesized from acryloyl chloride and docosyl alcohol. The average degree of polymerization was determined to be 21 by ¹H-NMR. The amount of DCA₂₁ immobilized onto silica was 18.0 wt%.

Poly(methyl acrylate) with a trimethoxysilyl group was also immobilized onto silica (Fuji Silysia Super Micro Bead silica gel 100A-5D). The preparation was completed using the method reported previously [11]. The average degree of polymerization was determined to be 21 by ¹H-NMR. The amount of immobilization onto silica was 21.3 wt%.

Ordinary octadecylated silica (ODS, C 13.7%) was prepared from Fuji Silysia Super Micro Bead silica gel 100A-5D with octadecyltrimethoxysilane and trimethylchlorosilane by the original procedure [9].

The silica-supported poly(octadecyl acrylate), Sil-ODA₂₄, poly(docosyl acrylate), Sil-DCA₂₁, poly(methyl acrylate), Sil-MA₂₁, and ODS were packed into stainless-steel columns (4.6 mm I.D. × 250 mm).

Diastomerizing Reagent and Optically Active Samples

(S)-(-)(2,3-Naphthalenedicarboximidyl)-propionyl fluoride (*Nip-F*) [4] was purchased from Dojin Chemical Co. (Japan) and used without further purification. (R)- and (S)-Phenylethylamines (PEA) were purchased from Nacalai Tesque (Japan) and used without further purification.

Diastomerization Procedure

PEA and triethylamine were added to a *Nip-F* methanol solution and then stirred for 60 min at room temperature. The resultant solution was used without any treatment and stocked at 5 °C.

Liquid Chromatography

The chromatograph included a JASCO 980 PU pump and a JASCO MD-910 UV-visible photodiode array detector. 5 μL of the sample (*Nip-PEA*) was injected through a Reodyne Model 7125 injector. Chromatography was carried out at flow-rate 0.5–1.0 mL min⁻¹. The retention factor (*k*) was determined by (t_e – t₀) / t₀, where t_e and t₀ are retention time of samples and methanol, respectively. The separation factor (*α*) was given by the ratio of retention factors.

Differential Scanning Calorimetry (DSC)

DSC thermograms of ODA₂₄ and DCA₂₁ and their silica-supported derivatives were obtained with a heating rate of 1 °C min⁻¹ using Seiko I & E SSC-580 with a DSC-10 instrument.

X-ray Diffraction

X-ray diffraction of ODA₂₄ and its silica-supported derivative were obtained by the reflection method (2θ-θ scan) and by the transmission method (edge view) with a Rigaku Denki RAD-IB X-ray diffractometer.

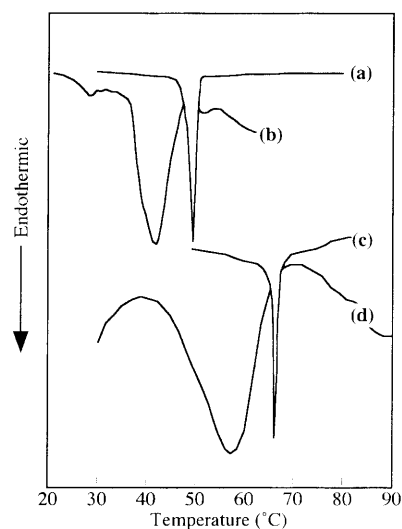


Figure 2. DSC thermograms of Sil-ODA₂₄ (a and b) and Sil-DCA₂₁ (c and d). The thermograms (b) and (d) were obtained in methanol-water (45:55).

Calculations

The *ab initio* MO/MP2 calculations were performed with Gaussian 94 [12]. The geometries of formaldehyde and benzene were fixed to be their MP2/6–31G* optimized equilibrium structures; R(C–O) = 1.2105 Å, R(C–H) = 1.1062 Å, and ∠(HCH) = 122.18° for formaldehyde, and (C–C) = 1.3991 Å and R(C–H) = 1.0864 Å for benzene. The binding energy of the formaldehyde-benzene complex was calculated with the MP2/6–311G (d, p). The basis set superposition error correction as a function of distance *R* between the carbon atom of formaldehyde and benzene plane, in which formaldehyde was moved perpendicular and/or parallel to the benzene plane (plane-to-plane interaction) with the orientation fixed to that of the optimized geometry.

The chemical structures of the diastereomers and terphenyls were estimated by Sony-Tektronix CACHE-mechanics with the MM2 option (until the energy changes were below 0.001 kcal mol⁻¹) and following CACHE-MOPAC Ver 6.00 with the PM3 option [13].

Results and Discussion

Physical States of Polymeric Organic Phases

Differential scanning calorimetry (DSC) showed that ODA₂₄ had a sharp endothermic peak around 49 °C (*T*_c, peak-

top temperature). A polarization microscopic observation of ODA₂₄ showed that a crystalline-to-isotropic phase transition was induced at a temperature around T_c . As support of this, X-ray analysis at room temperature provided a sharp diffraction with 4.15 Å at $2\theta = 21.4^\circ$.

Similar phase transition behavior was observed even after immobilization onto porous silica (Figure 2-a). The peak-top temperature (T_c) was 48 °C. The X-ray analysis at room temperature provided a diffraction with 4.16 Å at $2\theta = 21.3^\circ$. These values are very close to that of original ODA₂₄. This indicates that ODA₂₄ undergoes a crystalline-to-isotropic phase transition on silica and is in a crystalline state at room temperature. On the other hand, when the silica-supported ODA₂₄ (Sil-ODA₂₄) was placed in methanol-water (45:55), the peak-top temperature decreased from 48 to 42 °C with peak-broadening (Figure 2-b). This is attributable to a solvation effect but indicates that ODA₂₄ can still maintain a crystalline state at room temperature even in a chromatographic condition.

DSC study was applied to DCA₂₁ and MA₂₁ and their silica-supported derivatives (Sil-DCA₂₁ and Sil-MA₂₁). As shown in Figures 2-c and 2-d, the peak-top temperatures of DCA₂₁ and Sil-DCA₂₁ were detected at higher temperature ($T_{CS} = 66$ and 57 °C, respectively) than those of ODA₂₄ and Sil-ODA₂₄. These temperature increases indicate that DCA₂₁ can maintain a crystalline state at a higher temperature than ODA₂₄. On the other hand, MA₂₁ and Sil-MA₂₁ provide no endothermic peak at temperature range, 0–80 °C, indicating that MA₂₁ is always in an isotropic state in this temperature range and this is attributed to the fact that the MA moiety has no long-chain alkyl group for molecular orientation.

Temperature Dependence on Diastereomer Selectivity

(S)-(-)-(2,3-Naphthalenedicarboximidyl)propionyl fluoride (*Nip-F*) [4] was the commercially available diastereomerizing reagent used for chiral separation of phenylethylamines (PEA) as optically active amines. When the diastereomers of (R)- and (S)-PEAs with (*S*)-*Nip-F* were analyzed by ordinary octadecylated silica (ODS) in methanol-water (45:55) at 20 °C, a chromatogram shown in Figure 3-a was obtained. The separation factor

(α) was 1.06 and this value is very similar to that in the literature [4]. On the other hand, Sil-ODA₂₄ provided a better α value (1.12) under the same conditions as that of ODS (Figure 3-b). However, the α value decreased to 1.02 on increasing the temperature to 60 °C and a similar decrease (1.02 at 60 °C) was also observed with ODS. The detailed temperature dependencies of the separation factor were shown in Figure 4. The temperature dependence is more remarkable in Sil-ODA₂₄ than in ODS. Interestingly, the most remarkable decrease of α with increasing temperature was observed at temperatures below 45 °C. This unusual change of the separation factor becomes clear by plotting the temperature dependence on the selectivity enhancement against ODS. As shown in Figure 5, Sil-ODA₂₄ showed about two times higher selectivity than ODS at 0–40 °C but almost no enhancement was observed at temperatures above 45 °C. This critical temperature range (40–45 °C) for the enhancement change includes the phase transition temperature ($T_c = 42$ °C) of the immobilized organic phase (ODA₂₄). Therefore, it is clear that the distinct selectivity enhancement can be observed only at a temperature where ODA₂₄ is in a crystalline state.

Effect of Mobile Phase

Figure 6 shows the temperature dependencies of the separation factor on the mobile phase composition. The composition of the mobile phases were chosen so that the retention factors would be similar at 45 °C. As shown in Figure 6, the α value with acetonitrile was smaller than with methanol. Interestingly, the α value (= 1.10) at 0 °C in the acetonitrile-containing phase is almost the same as that in ODS at 0 °C ($\alpha = 1.09$). On the other hand, DSC did not show significant difference in the phase transition behavior. These results indicate that acetonitrile works as an inhibitor for diastereomer selectivity regardless of the fact that the ODA₂₄ phase is in a highly-oriented state.

Effect of Long-Chain Alkyl Groups on the Stationary Phase

The organic phase of Sil-MA₂₁ has no long chain-alkyl group while the chemical structure except for the side chain length

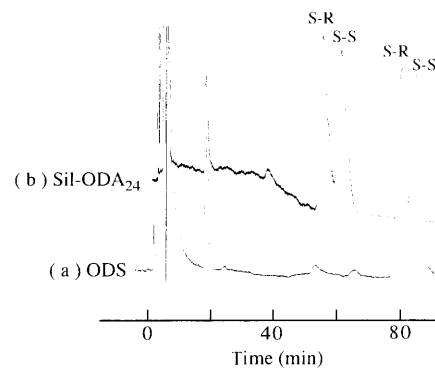


Figure 3. Typical chromatograms for the mixture of (*S*)-*Nip*-(*S*)-PEA and (*S*)-*Nip*-(*R*)-PEA with Sil-ODA₂₄ and ODS columns at 20 °C. Mobile phase: methanol-water (45:55).

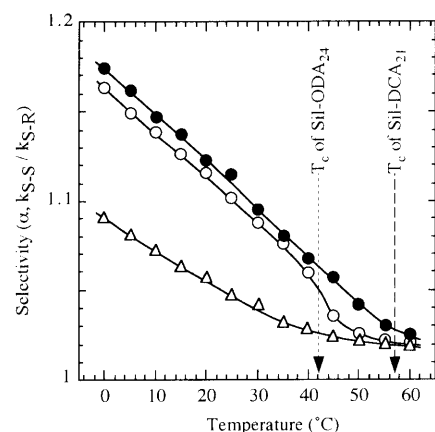


Figure 4. Temperature dependencies of the separation factor (α) determined by the ratio of the retention factors between (*S*)-*Nip*-(*S*)-PEA and (*S*)-*Nip*-(*R*)-PEA with Sil-ODA₂₄ (○), Sil-DCA₂₁ (●) and ODS (△). Mobile phase: methanol-water (45:55).

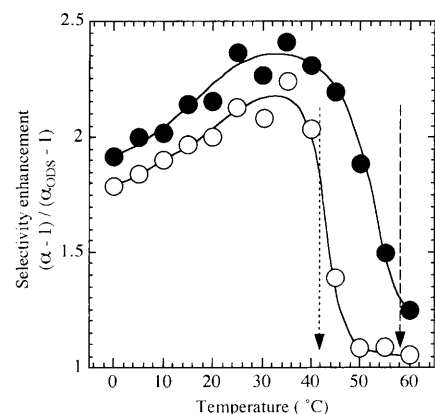


Figure 5. Selectivity enhancement due to Sil-ODA₂₄ (○) and Sil-DCA₂₁ (●) against ODS.

is similar to ODA₂₄. Therefore, no phase transition was observed. Also no diastereomer separation ($\alpha \sim 1.0$ at 0–60 °C) was observed for the *Nip*-PEA diastereomers. To clarify the importance of the long-chain alkyl groups of the polymeric organic phase, poly(docosyl acrylate)

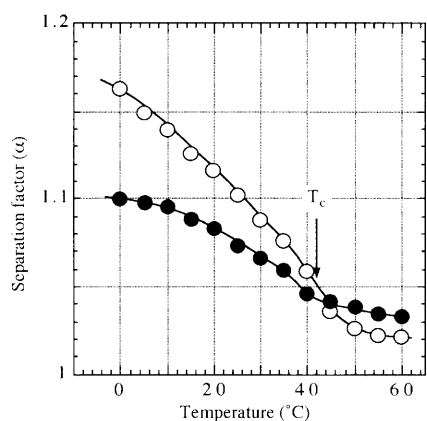


Figure 6. Effect of the mobile phase component on temperature dependencies of the separation factor (α) with Sil-ODA₂₄. Mobile phase: (○), methanol-water = 45:55; (●), acetonitrile-water = 33:67.

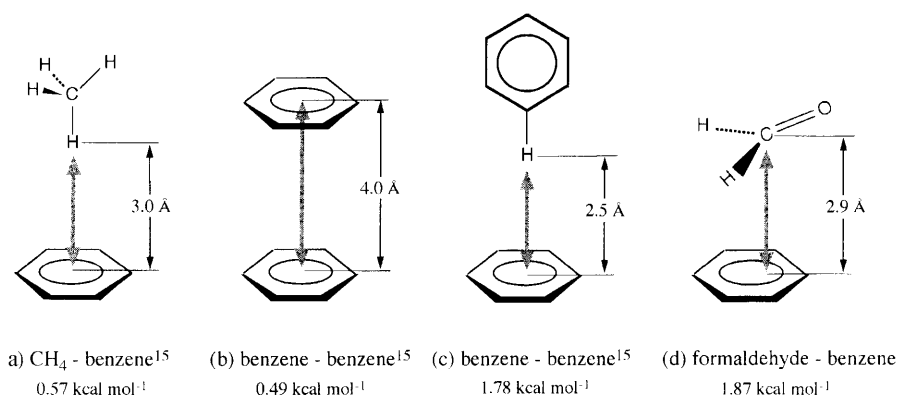


Figure 7. Proposed structures and energies of methane-benzene¹⁵ (a), benzene-benzene¹⁵ (b and c) formaldehyde-benzene (d) complexes estimated by *ab initio* study.

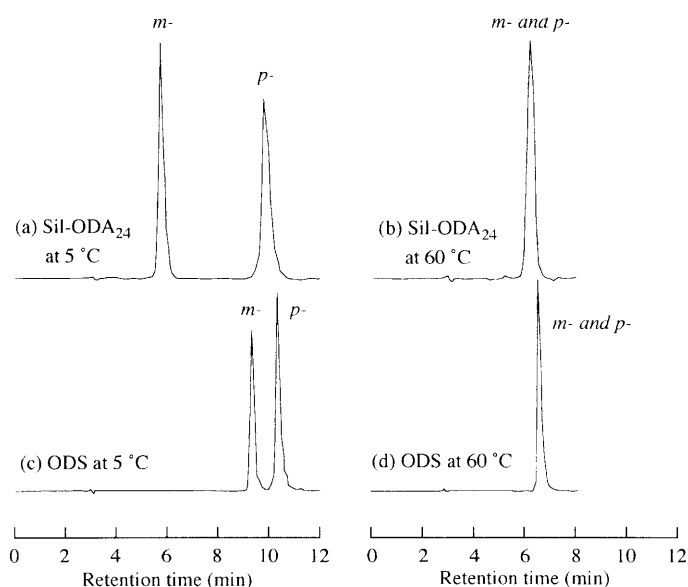


Figure 8. Typical chromatograms of the mixture of *m*- and *p*-terphenyls with Sil-ODA₂₄ and ODS columns at 5 and 60 °C. T_c in the figure is a peak-top temperature which was determined in methanol-water (9:1). Mobile phase: methanol-water (9:1).

(DCA) with longer alkyl chains than ODA was prepared and immobilized onto silica. As shown in Figure 4, silica-supported DCA₂₁ (Sil-DCA₂₁) also showed temperature dependence on the diastereomer separation. The selectivities at temperature below 45 °C were slightly higher than those of Sil-ODA₂₄. DSC showed that the silica-supported DCA₂₁ underwent a crystal-to-isotropic phase transition at temperature around 57 °C in a methanol-water (45:55) dispersion. This good relation between the selectivity increase and the phase transition temperature becomes clear by comparing the selectivity enhancement. As shown in Figure 5, the temperature range (50–60 °C)

which shows a selectivity change is higher than in ODA₂₄ (40–50 °C).

Discussion on the Mechanism for Enhanced Selectivity

In our recent study, we have reported that ODA_n which is in a crystalline state showed remarkable molecular planarity recognition for polyaromatic hydrocarbons [9, 10] and this ability is through a carbonyl- π interaction [9, 11, 14]. According to the present calculations with formaldehyde-benzene complexes as a model system, the carbonyl-benzene interaction (1.87 kcal mol⁻¹ at 2.9 Å, Figure 7-d) is

more effective compared with CH₄-benzene (0.57 kcal mol⁻¹ at $R = 3.0$ Å, Figure 7-a) and benzene-benzene complexes (0.49 or 1.78 kcal mol⁻¹ at $R = 4.0$ or 2.5 Å, Figures 7-b and 7-c) [15]. Therefore, we assume that the diastereomer selectivities of Sil-ODS₂₄ and Sil-DCA₂₁ are brought about with a carbonyl- π interaction as well as a highly-oriented state. As support of this, use of acetonitrile as a mobile phase suppressed the selectivity enhancement. This is because acetonitrile possesses π -electrons. These π -electrons probably inhibit the interaction between the naphthyl π -electrons of the diastereomers and the carbonyl groups of ODA₂₄ and DCA₂₁. A similar inhibition effect was observed with acetone but not with 2-propanol. On the other hand, Sil-MA₂₁ did not show any effective diastereomer selectivity regardless of the fact that an MA₂₁ moiety has a lot of carbonyl groups per molecule. Therefore, we conclude that only carbonyl groups on a highly-oriented structure can enhance the diastereomer selectivity.

To explain why the highly-oriented structure (crystalline state) of the polymeric phase can enhance the selectivity, we carried out the following additional experiments: Figure 8 shows chromatograms for the mixture of *m*- and *p*-terphenyls at 5 °C. It is clear that Sil-ODA₂₄ showed much larger selectivity than ODS. The difference can be emphasized by the temperature-selectivity plots. As shown in Figure 9, the selectivity in the separation between *p*- and *m*-terphenyls increased at temperature below 40 °C although ODS always showed low selectivity (below 1.1) regardless of temperature change. The dis-

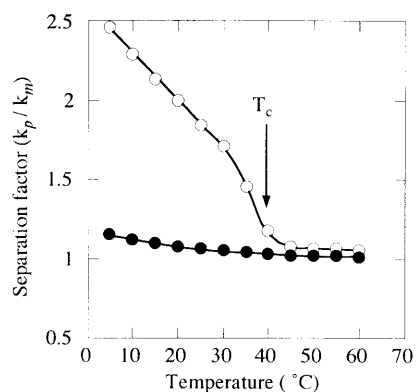


Figure 9. Temperature dependencies on the separation factor (a) between p - and m -terphenyls with Sil-ODA₂₄ and ODS columns. Mobile phase: methanol-water (9:1).

inct bending point in Figure 9 is closely related to the phase transition temperature of Sil-ODA₂₄. To explain this selectivity enhancement, we propose the molecular-linearity recognition mechanism in this paper as schematically illustrated in Figure 10. This assumption can be defined as follows: if carbonyl groups as π - π interaction sources are highly-oriented as shown in Figure 10-a, multiple interactions will occur more effectively with a linear and planar compound such as p -terphenyl than with bending and twisted compounds such as m - and o -terphenyls. On the other hand, carbonyl groups in an isotropic state (Figure 10-b) will not provide such special conformation. On this view point, it should be noted that the S-S isomer is more planar than the S-R isomer as shown in Figure 10. Therefore, we conclude that the enhanced selectivities derived from Sil-ODA₂₄ and Sil-DCA₂₁ are brought by the molecular-planarity and linearity recognition ability.

In conclusion, we have hypothesized that carbonyl- π interaction on a highly-oriented structure enhances diastereomer selectivity. We have described the first example with (S)-(-)(2,3-naphthalenedicarboximidyl)propionyl fluoride. Fortunately, almost all diastereomerizing reagents have π -electron-rich aromatic rings per molecule. Therefore, our new finding will be expanded furthermore.

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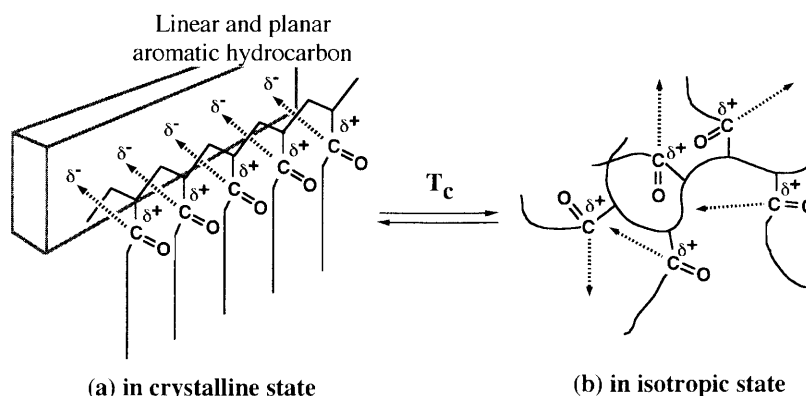
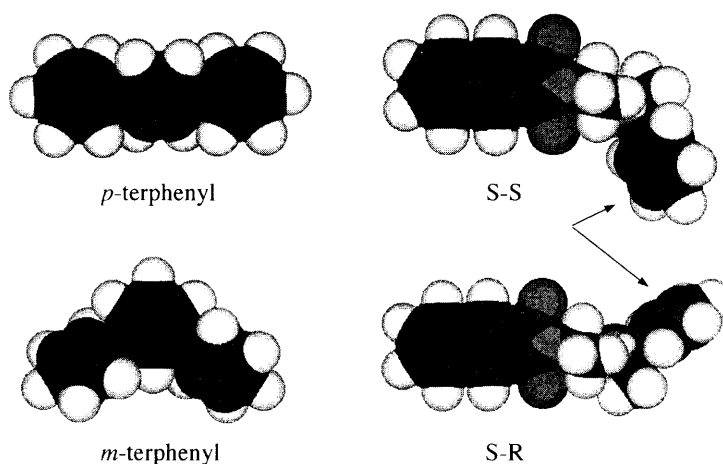


Figure 10. Schematic illustration of molecular-linearity recognition through multiple carbonyl- π interactions. The figures indicate that a planar and linear aromatic hydrocarbon provides more effective interaction area with a carbonyl-containing polymer which is in a highly-oriented state (a) than in an isotropic state (b). The estimated structures of terphenyl were obtained by MOPAC simulation. When the naphthyl planes are placed in parallel on the paper, the phenyl plane (indicated by a solid arrow) in the S-R isomer shows more twisted conformation than in the S-S isomer.

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