J|A|C|S
ARTICLES
Published on Web 12/28/2002

Detection and Amplification of a Small Enantiomeric Imbalance in α-Amino Acids by a Helical Poly(phenylacetylene) with Crown Ether Pendants

Ryuji Nonokawa and Eiji Yashima*

Contribution from the Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan

Received August 30, 2002; E-mail: yashima@apchem.nagoya-u.ac.jp

Abstract: We have designed a novel stereoregular poly(phenylacetylene) bearing the bulky crown ether as the pendant (poly-**1**) for the amino acid binding site. The polymer forms a one-handed helix upon complexation with L-amino acid perchlorates, and the complexes exhibit an induced circular dichroism (ICD) with the same Cotton effect signs in the polymer backbone region through a significant cooperative interaction. Poly-**1** is highly sensitive to the amino acid chirality and can detect an extremely small enantiomeric imbalance in α-amino acids (less than 0.005% enantiomeric excess of alanine, for example).

Introduction

The origin of biomolecular homochirality such as L-amino acids and D-sugars in living systems[1] has been postulated to be caused by physical forces such as magnetochiral anisotropy,[2] circular polarized light (CPL),[3] and an electroweak interaction.[1,4] However, the enantiomeric excess (ee) of the amino acids induced by these forces is quite small, and the detection is very difficult by conventional analytical methods, so that the development of detecting methods[5] that are highly sensitive to a small imbalance of the amino acids chirality, that also require universal availability to all chiral amino acids, is valuable in the area of chemistry, physics, astronomy, and geology.

In earlier studies, we reported the induction of helicity in achiral poly(phenylacetylene)s bearing functional groups, such as carboxy,[6] amino,[7] boronate,[8] and phosphonate groups.[9] In the presence of optically active compounds capable of interacting with the polymer's functional groups, a dynamic, one-handed macromolecular helicity is induced in the polymers, resulting in a characteristic, induced circular dichroism (ICD) in the UV−visible region.[10] A similar drastic CD change was also observed in an optically active poly(phenylacetylene) having a β-cyclodextrin upon inclusion complexation with chiral and achiral guest molecules.[11] We now designed and synthesized a *cis*−*transoidal* poly(phenylacetylene) consisting of a chromophoric polyacetylene backbone and a bulky crown ether as the pendant for the amino acid binding site (poly-**1**; Figure 1).[12] Crown ethers are known to form stable complexes with amino acids,[13] and, as shown below, the amino acid derived chiral information is transmitted to the polymer backbone leading to an excess of the one-handed helical sense, which is the source of the observed optical activity (ICD) (Figure 1).

We further report that an extremely small enantiomeric imbalance in α-amino acids can be detected by a significant amplification of the amino acid chirality using poly-**1**, through a cooperative nonbonding interaction. In the literature, a high amplification chirality has been reported by various means, for example, via polymerization,[14] supramolecular aggregation,[15]

(1) Reviews: (a) Bonner, W. A. *Top. Stereochem.* **1988**, *18*, 1−96. (b) Keszthelyi, L. *Q. Rev. Biophys.* **1995**, *28*, 473−507. (c) Avalos, M.; Babiano, R.; Cintas, P.; Jiménez, J. L.; Palacios, J. C. *Tetrahedron: Asymmetry* **2000**, *11*, 2845−2874.
(2) Rikken, G. L. J. A.; Raupach, E. *Nature* **2000**, *405*, 932−935.
(3) (a) Rau, H. *Chem. Rev.* **1983**, *83*, 535−547. (b) Inoue, Y. *Chem. Rev.* **1992**, *92*, 741−770. (c) Bailey, J.; Chrysostomou, A.; Hough, J. H.; Gledhill, T. M.; McCall, A.; Clark, S.; Ménard, F.; Tamura, M. *Science* **1998**, *281*, 672−674. (d) Green, M.; Selinger, J. V. *Science* **1998**, *282*, 880−881. (e) Bailey, J. *Origins Life Evol. Biosphere* **2001**, *31*, 167−183.
(4) (a) Mason, S. F. *Nature* **1984**, *311*, 19−23. (b) Avalos, M.; Babiano, R.; Cintas, P.; Jiménez, J. L.; Palacios, J. C.; Barron, L. D. *Chem. Rev.* **1998**, *98*, 2391−2404. (c) Service, R. F. *Science* **1999**, *286*, 1282−1283. (d) Berger, R.; Quack, M. *ChemPhysChem* **2000**, 57−60. (e) Berger, R.; Gottselig, M.; Quack, M.; Willeke, M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4195−4198.
(5) Pizzarello, S.; Cronin, J. R. *Nature* **1998**, *394*, 236.
(6) (a) Yashima, E.; Matsushima, T.; Okamoto, Y. *J. Am. Chem. Soc.* **1995**, *117*, 11596−11597. (b) Yashima, E.; Matsushima, T.; Okamoto, Y. *J. Am. Chem. Soc.* **1997**, *119*, 6345−6359. (c) Yashima, E.; Goto, H.; Okamoto, Y. *Polym. J.* **1998**, *30*, 69−71. (d) Yashima, E.; Maeda, K.; Okamoto, Y. *Nature* **1999**, *399*, 449−451. (e) Saito, M. A.; Maeda, K.; Onouchi, H.; Yashima, E. *Macromolecules* **2000**, *33*, 4616−4618. (f) Maeda, K.; Goto, H.; Yashima, E. *Macromolecules* **2001**, *34*, 1160−1164.
(7) (a) Yashima, E.; Maeda, Y.; Okamoto, Y. *Chem. Lett.* **1996**, 955−956. (b) Yashima, E.; Maeda, Y.; Matsushima, T.; Okamoto, Y. *Chirality* **1997**, *9*, 593−600.
(8) (a) Yashima, E.; Nimura, T.; Matsushima, T.; Okamoto, Y. *J. Am. Chem. Soc.* **1996**, *118*, 9800−9801. (b) Kawamura, H.; Maeda, K.; Okamoto, Y.; Yashima, E. *Chem. Lett.* **2001**, 58−59.
(9) Onouchi, H.; Maeda, K.; Yashima, E. *J. Am. Chem. Soc.* **2001**, *123*, 7441−7442.
(10) Yashima, E. *Anal. Sci.* **2002**, *18*, 3−6.
(11) Yashima, E.; Maeda, K.; Sato, O. *J. Am. Chem. Soc.* **2001**, *123*, 8159−8160.
(12) Polyacetylenes bearing a crown ether moiety have been prepared, and their metal-binding properties and morphology were investigated. (a) Kakuchi, T.; Matsunami, S.; Kamimura, H.; Ishii, F.; Uesaka, T.; Yokota, K. *J. Polym. Sci., Part A: Polym. Chem.* **1995**, *33*, 1431−1436. (b) Cheuk, K. K. L.; Lam, J. W. Y.; Cha, J. A. K.; Tang, B. Z. *Polym. Prepr.* **2000**, *41(1)*, 131−132.
(13) (a) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1009−1020. (b) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89−112.
(14) Jha, S. K.; Cheon, K.-S.; Green, M. M.; Selinger, J. V. *J. Am. Chem. Soc.* **1999**, *121*, 1665−1673.

10.1021/ja028348c CCC: $25.00

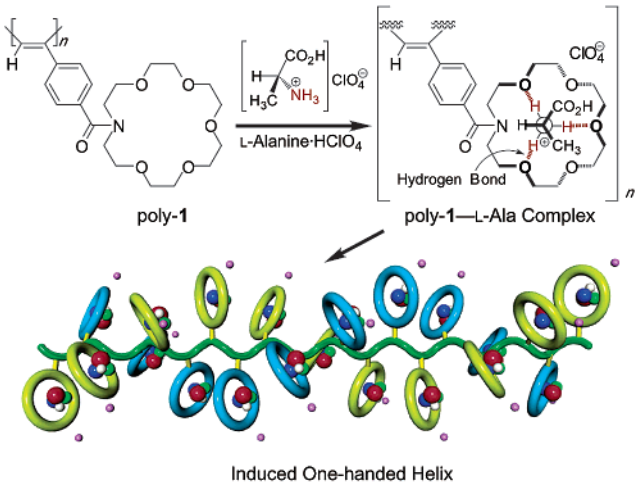


Figure 1. Schematic representation of the macromolecular helicity induction on poly-**1** upon complexation with L-alanine. The crown ether pendants, represented by yellow and blue rings for clarity, arrange in a helical array with a predominant screw-sense along the polymer backbone (bottom). The helix-sense of poly-**1** is tentative.

autocatalytic asymmetric synthesis,[16] or by asymmetric photoreaction using CPL.[17] Yet the present system differs in a fundamental manner from this prior work in requiring no further chemical reactions and derivatization and can provide a reliable methodology for the rapid detection and identification of small enantiomeric imbalances in amino acids and related chiral molecules such as amino alcohols and even isovaline extracted from meteorites[18] and also those produced by CPL.[19]

Results and Discussion

Synthesis and Helicity Induction of Poly-1 with Chiral Amino Acids and Amino Alcohols. *Cis*−*transoidal* poly-**1** was prepared by polymerization of the corresponding monomer with a rhodium catalyst in a method similar to that previously reported.[6−9] The number average molecular weight (M_n) was estimated to be 19.7×10^4 ($M_w/M_n = 2.6$; degree of polymerization (DP) = 503) as determined by size exclusion chromatography (SEC) with polystyrene standards using tetrahydrofuran (THF) containing 0.1 wt % tetra-*n*-butylammonium bromide as the eluent. The ^{1}H NMR spectrum of poly-**1** in $CDCl_3$ showed a sharp singlet centered at 5.84 ppm, due to the main chain protons, indicating that the polymer possesses a highly *cis*−*transoidal*, stereoregular structure.[20]

The typical CD and absorption spectra of poly-**1** in the presence of the simple amino acid, L- and D-alanine (Ala; 2 equiv to monomer units of poly-**1**) complexed with $HClO_4$ in pure acetonitrile, are shown in Figure 2A. The complexes showed mirror images of split-type intense ICDs. The ICD magnitude slightly increased with the decreasing temperature. The CD titration using L-Ala showed that the CD intensity increased with the increasing concentration of L-Ala and reached an almost constant value at 1 equiv of L-Ala at −10 °C (Figure 2B). Plots of the CD intensities of the second Cotton ($\Delta\epsilon_{2nd}$) of poly-**1** as a function of concentration of L-Ala gave a saturation binding isotherm. The Hill plot analysis of the data resulted in the apparent binding constants (*K*s) of 1.8×10^4 and 2.6×10^4 at 25 and −10 °C, respectively.[21] We note that at −10 °C, 0.1 equiv of L-Ala induced an almost one-handed helix, and poly-**1** exhibited an apparent ICD even with 0.01 equiv of L-Ala, indicative of a strong chiral amplification with cooperative interaction in the pendants. That is, a very small chiral bias in the monomeric crown ether units of poly-**1** complexed with L-Ala is amplified to induce the same helix on the major free monomeric crown ether units. This result indicates that the poly-**1** may be acting analogously to the polyisocyanates in their stiff helical character with alternating left- and right-handed helical segments separated by rarely occurring helix reversals.[22] Application of chiral information is then acted on cooperatively to induce an excess helical sense upon complexation with L- or D-Ala.[23] Similar helicity induction on optically inactive polymers and oligomers through intermolecular chiral interactions has been reported.[24]

The assay of 19 of the common L-amino acids (Ala, Arg, Asn, Asp, Cys, Gln, Glu, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, Trp, Val) produced intense ICDs with the same Cotton effect signs ($\Delta\epsilon_{2nd} = -10$ to -17) even at 25 °C; only the secondary amino acid (L-Pro) showed a weak ICD ($\Delta\epsilon_{2nd} = -0.29$ at 25 °C and -0.79 at 0 °C) (Table 1). The lower limit of detection of L-Ala with concentrated poly-**1** solution (61 μg, 5 mg/mL) was examined, and it was found that only

(15) (a) Hirschberg, J. H. K. K.; Brunsveld, L.; Ramzi, A.; Vekemans, J. A. J. M.; Sijbesma, R. P.; Meijer, E. W. *Nature* **2000**, *407*, 167−170. (b) Prins, L. J.; Timmerman, P.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2001**, *123*, 10153−10163.
(16) (a) Shibata, T.; Yamamoto, J.; Matsumoto, N.; Yonekubo, S.; Osanai, S.; Soai, K. *J. Am. Chem. Soc.* **1998**, *120*, 12157−12158. (b) Soai, K.; Shibata, T.; Sato, I. *Acc. Chem. Res.* **2000**, *33*, 382−390.
(17) (a) Huck, N. P. M.; Jager, W. F.; de Lange, B.; Feringa, B. L. *Science* **1996**, *273*, 1686−1688. (b) Feringa, B. L.; van Delden, R. A. *Angew. Chem., Int. Ed.* **1999**, *38*, 3418−3438.
(18) (a) Kvenvolden, K.; Lawless, J.; Pering, K.; Peterson, E.; Flores, J.; Ponnamperuma, C.; Kaplan, I. R.; Moore, C. *Nature* **1970**, *228*, 923−926. (b) Bada, J. L. *Philos. Trans. R. Soc. London, Ser. B* **1991**, *333*, 349−358. (c) Cronin, J. R.; Pizzarello, S. *Science* **1997**, *275*, 951−954. (d) Engel, M. H.; Macko, S. A. *Nature* **1997**, *389*, 265−268. (e) Bada, J. L.; Glavin, D. P.; McDonald, G. D.; Becker, L. *Science* **1998**, *279*, 362−365. (f) Podlech, J. *Angew. Chem., Int. Ed.* **1999**, *38*, 477−478.
(19) Nishino, H.; Kosaka, A.; Hembury, G. A.; Shitomi, H.; Onuki, H.; Inoue, Y. *Org. Lett.* **2001**, *3*, 921−924.
(20) (a) Simionescu, C.; Dumitrescu, S.; Percec, V. *J. Polym. Sci., Polym. Symp.* **1978**, *64*, 209−227. (b) Furlani, A.; Napoletano, C.; Russo, M. V.; Feast, W. J. *Polym. Bull.* **1986**, *16*, 311−317. (c) Kishimoto, Y.; Eckerle, P.; Miyatake, T.; Kainosho, M.; Ono, A.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1999**, *121*, 12035−12044.
(21) Connors, K. A. *Binding Constants*; John Wiley: New York, 1987.
(22) (a) Li, J.; Schuster, G. B.; Cheon, K.-S.; Green, M. M.; Selinger, J. V. *J. Am. Chem. Soc.* **2000**, *122*, 2603−2612. For reviews, see: (b) Green, M. M.; Peterson, N. C.; Sato, T.; Teramoto, A.; Cook, R.; Lifson, S. *Science* **1995**, *268*, 1860−1866. (c) Green, M. M.; Park, J.-W.; Sato, T.; Teramoto, A.; Lifson, S.; Selinger, R. L. B.; Selinger, J. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3138−3154.
(23) We measured the viscosity of poly-**1** with or without L-Ala to investigate a change in rigidity of the polymer upon complexation with L-Ala. Poly-**1** showed a linear relationship in the Huggins plot, and the intrinsic viscosity was estimated to be 2.00 dL/g. However, in the presence of L-Ala, the intrinsic viscosity increased to 3.56, which was 1.8 times larger than that of the poly-**1**. Under the present experimental conditions, the poly-**1** solutions containing L-Ala showed a full ICD at 25 °C. These results suggest that poly-**1** becomes more stiff than the poly-**1** upon complexation with L-Ala (see Supporting Information).
(24) (a) Schlitzer, D. S.; Novak, B. M. *J. Am. Chem. Soc.* **1998**, *120*, 2196−2197. (b) Norris, I. D.; Kane-Maguire, L. A. P.; Wallace, G. G. *Macromolecules* **1998**, *31*, 6529−6533 (c) Maeda, K.; Yamamoto, N.; Okamoto, Y. *Macromolecules* **1998**, *31*, 5924−5926. (d) Nielsen, P. E. *Acc. Chem. Res.* **1999**, *32*, 624−630. (e) Yashima, E.; Maeda, K.; Yamanaka, T. *J. Am. Chem. Soc.* **2000**, *122*, 7813−7814. (f) Prince, R. B.; Barnes, S. A.; Moore, J. S. *J. Am. Chem. Soc.* **2000**, *122*, 2758−2762. (g) Ishikawa, M.; Maeda, K.; Yashima, E. *J. Am. Chem. Soc.* **2002**, *124*, 7448−7458. For reviews of helical polymers and oligomers (foldamers), see: (h) Okamoto, Y.; Nakano, T. *Chem. Rev.* **1994**, *94*, 349−372. (i) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180. (j) Srinivasarao, M. *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 370−376. (k) Fujiki, M. *Macromol. Rapid Commun.* **2001**, *22*, 539−563. (l) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4011. (m) Okamoto, Y.; Nakano, T. *Chem. Rev.* **2001**, *101*, 4013−4038. (n) Conrnelissen, J. J. L. M.; Rowan, A. E.; Nolte, R. J. M.; Sommerdijk, N. A. J. M. *Chem. Rev.* **2001**, *101*, 4039−4070. (o) Brunsveld, L.; Folmer, B. J. B.; Meijer, E. W.; Sijbesma, R. P. *Chem. Rev.* **2001**, *101*, 4071−4097.

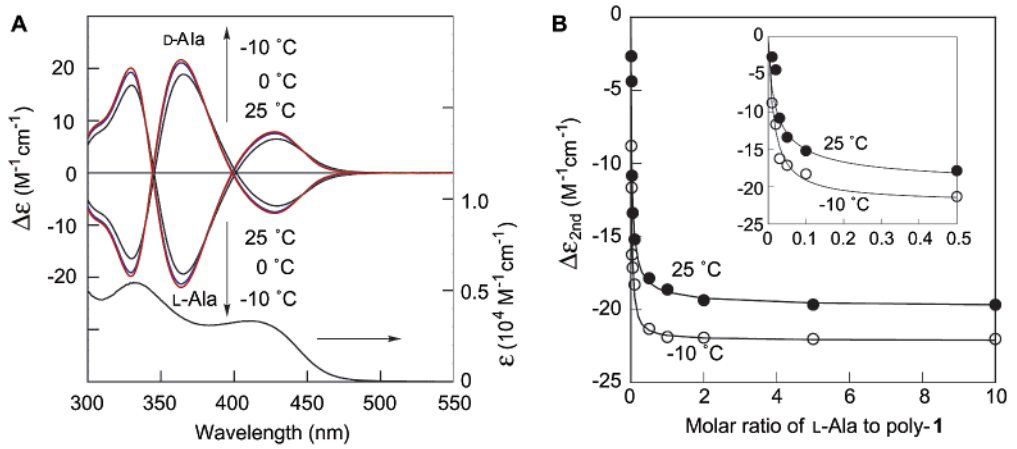


Figure 2. Helicity induction on poly-**1** with Ala. (A) CD spectra (molar ellipticity, $\Delta\epsilon$) of poly-**1** with L- and D-Ala·$HClO_4$ in acetonitrile at 25, 0, and −10 °C. Absorption spectrum (molar absorptivity, ϵ) of poly-**1** with L-Ala·$HClO_4$ at 25 °C is also shown. The concentration of poly-**1** is 1.0 mg (2.6 μmol monomer units)/mL. (B) Titration curves of poly-**1** ($\Delta\epsilon_{2nd}$) with L-Ala·$HClO_4$ in acetonitrile at 25 and −10 °C. Here $\Delta\epsilon_{2nd}$ indicates the ICD intensity at the second Cotton (365 nm). Inset shows expanded detail of the titration curves. Curves in the plots were the calculated ones using $K = 1.8 \times 10^4$ and 2.6×10^4 at 25 and −10 °C, respectively.

Table 1. Signs and Difference in Exciton Coefficient of the Second Cotton ($\Delta\epsilon_{2nd}$) for the Complexes of Poly-**1** with Amino Acids or Amino Alcohols in CH_3CN−1 N $HClO_4$ (97.2/2.8, v/v)[a]

		second Cotton [λ (nm) and $\Delta\epsilon_{2nd}$ ($M^{-1}cm^{-1}$)]		
guest	sign	25 °C λ ($\Delta\epsilon$)	10 °C λ ($\Delta\epsilon$)	0 °C λ ($\Delta\epsilon$)
		amino acid		
L-Ala	−	368 (17.39)	367 (18.55)	367 (19.16)
L-Ala[b]	−	364 (19.50)	363 (20.69)	363 (21.40)
D-Ala	+	366 (17.25)	367 (18.36)	367 (19.03)
L-Asn[c]	−	370 (10.69)	371 (15.05)	371 (16.30)
L-Cys	−	369 (16.83)	369 (18.17)	369 (18.85)
L-Gln	−	370 (16.33)	370 (17.62)	370 (18.30)
L-Ile	−	370 (16.72)	370 (18.08)	369 (18.80)
L-Leu	−	370 (17.43)	369 (18.64)	369 (19.27)
L-Met	−	369 (17.72)	368 (18.95)	368 (19.58)
L-Phe	−	370 (16.96)	370 (18.18)	370 (18.81)
L-Pro	−	373 (0.29)	372 (0.65)	372 (0.79)
L-Ser	−	369 (16.63)	368 (17.89)	368 (18.49)
L-Thr	−	371 (16.03)	371 (17.57)	372 (18.35)
L-Trp	−	370 (17.38)	370 (18.75)	370 (19.53)
L-Tyr	−	371 (15.04)	371 (16.25)	371 (16.88)
L-Val	−	370 (16.63)	370 (18.01)	370 (18.74)
L-Asp[c]	−	370 (14.83)	370 (16.88)	370 (17.69)
L-Glu[c]	−	369 (17.34)	369 (18.88)	369 (19.65)
L-Arg[d]	−	371 (12.16)	371 (14.79)	*e*
L-His[d]	−	371 (10.08)	372 (13.60)	*e*
L-Lys[d]	−	371 (12.06)	371 (14.81)	*e*
L-Isovaline	−	371 (1.87)	371 (3.27)	370 (3.93)
		amino alcohol		
(*S*)-**2**	−	371 (6.46)	371 (13.32)	371 (15.76)
(*S*)-**3**	−	371 (5.55)	371 (11.39)	371 (14.09)
(*R*)-**3**	+	371 (5.75)	371 (11.43)	371 (13.90)
(*S*)-**4**	−	371 (4.87)	371 (11.96)	371 (15.00)
(*S*)-**5**	−	371 (7.05)	371 (13.51)	371 (15.47)

[a] The concentration of poly-**1** is 1.0 mg/mL, and the molar ratio of a guest to monomer units of poly-**1** is 10; [$HClO_4$]/[guest] = 1.1. [b] In CH_3CN. [c] In CH_3CN−1 N $HClO_4$−water (95.0/2.8/2.2, v/v) and [amino acid]/[poly-**1**] = 5. [d] In CH_3CN−1 N $HClO_4$−water (92.5/2.8/4.7, v/v) and [amino acid]/[poly-**1**] = 5. [e] It could not be measured because the solution became turbid.

70 ng of L-Ala, corresponding to 0.005 equiv of the monomer units of poly-**1**, was enough to produce an ICD at 25 °C. These results belong to the class of sergeants and soldiers effects widely observed in helical polymers[14,22,25] and supramolecular helical arrays, with the amino acids acting as the sergeants in the present system.[15,26]

Although a number of receptor molecules for amino acids have been prepared, most of these receptor molecules showed molecular and chiral recognition for protected amino acids, and host−guest systems capable of recognizing free amino acids are still rare. This indicates that for detecting amino acid chirality, poly-**1** is indeed among the most sensitive and useful synthetic receptors.[27]

Poly-**1** also responded to chiral amino alcohols (Chart 1), and the complexes exhibited similar ICDs in their patterns (Table 1). Here also the ICD intensity considerably increased with the decreasing temperature. The amino alcohols ((*S*)-**2**−(*S*)-**5**) derived from the L-amino acids, L-phenylalanine, L-phenylglycine, L-leucine, and L-alanine, respectively, exhibited the same Cotton effect signs as the L-amino acids, indicating that poly-**1** will show ICDs with the same Cotton effect signs even if the amino acids contain the amino alcohols derived from the amino acids with the same configuration as an impurity.

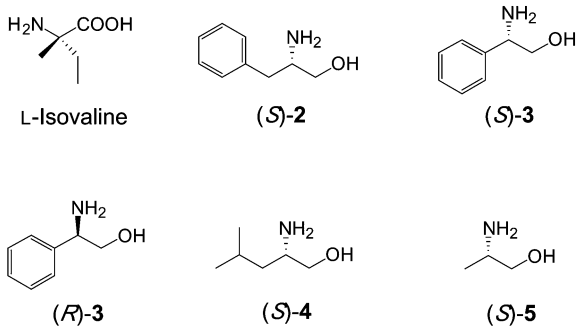
Chiral Amplification. We then investigated the changes in the ICD intensity with respect to the ee of Ala (Figure 3) and

(25) (a) Green, M. M.; Reidy, M. P.; Johnson, R. D.; Darling, G.; O'Leary, D. J.; Wilson, G. *J. Am. Chem. Soc.* **1989**, *111*, 6452−6454. (b) Yashima, E.; Huang, S.; Matsushima, T.; Okamoto, Y. *Macromolecules* **1995**, *28*, 4184−4193. (c) Maeda, K.; Okamoto, Y. *Macromolecules* **1998**, *31*, 1046−1052. (d) Takei, F.; Onitsuka, K.; Takahashi, S. *Polym. J.* **2000**, *32*, 524−526. (e) Nomura, R.; Fukushima, Y.; Nakako, H.; Masuda, T. *J. Am. Chem. Soc.* **2000**, *122*, 8830−8836. (f) Toyoda, S.; Fujiki, M. *Macromolecules* **2001**, *34*, 640−644.

(26) For intermolecular sergeants and soldiers effects in supramolecular helical arrays, see: (a) Palmans, A. R. A.; Vekemans, J. A. J. M.; Havinga, E. E.; Meijer, E. W. *Angew. Chem., Int. Ed. Engl*. **1997**, *36*, 2648−2651. (b) Langeveld-Voss, B. M. W.; Waterval, R. J. M.; Janssen, R. A. J.; Meijer, E. W. *Macromolecules* **1999**, *32*, 227−230. (c) Brunsveld, L.; Meijer, E. W.; Prince, R. B.; Moore, J. S. *J. Am. Chem. Soc.* **2001**, *123*, 7978−7984. (d) Schenning, A. P. H. J.; Kilbinger, A. F. M.; Biscarini, F.; Cavallini, M.; Cooper, H. J.; Derrick, P. J.; Feast, W. J.; Lazzaroni, R.; Leclère, P.; McDonell, L. A.; Meijer, E. W.; Meskers, S. C. J. *J. Am. Chem. Soc.* **2002**, *124*, 1269−1275. For intramolecular sergeants and soldiers effects in supramolecular helical arrays, see: (e) Fiesel, R.; Scherf, U. *Acta Polym.* **1998**, *49*, 445−449. (f) Prince, R. B.; Moore, J. S.; Brunsveld, L.; Meijer, E. W. *Chem.-Eur. J.* **2001**, *7*, 4150−4154.

(27) For recent examples of chirality recognition of protected amino acids in organic media, see: (a) Huang, X.; Rickman, B. H.; Borhan, B.; Berova, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1998**, *120*, 6185−6186. (b) Zahn, S.; Canary, J. W. *Org. Lett.* **1999**, *1*, 861−864. (c) Mizutani, T.; Sakai, N.; Yagi, S.; Takagishi, T.; Kitagawa, S.; Ogoshi, H. *J. Am. Chem. Soc.* **2000**, *122*, 748−749. (d) Shinkai, S.; Ikeda, M.; Sugasaki, A.; Takeuchi, M. *Acc. Chem. Res.* **2001**, *34*, 494−503. (e) Canary, J. W.; Holmes, A. E.; Liu, J. *Enantiomer* **2001**, *6*, 181−188. For recent examples of chiral or chirality recognition of free amino acids, see ref 9 and: (f) Tsukube, H.; Shinoda, S.; Uenishi, J.; Kanatani, T.; Itoh, H.; Shiode, M.; Iwachido, T.; Yonemitsu, O. *Inorg. Chem.* **1998**, *37*, 1585−1591. (g) Leipert, D.; Nopper, D.; Bauser, M.; Gauglitz, G.; Jung, G. *Angew. Chem., Int. Ed.* **1999**, *38*, 3308−3311.

Chart 1. Structures of L-Isovaline and Chiral Amino Alcohols



observed an extremely strong chiral amplification (positive nonlinear effect) despite the noncovalent bonding interaction in this system. Even a 5% ee of Ala gave rise to the full ICD as induced by pure L-Ala. Complexation of a slight excess of one enantiomeric Ala with the pendant groups of the polymer led to an excess of the helical sense preferred by the majority enantiomer.[22] Similar strong chiral amplifications were also observed for Leu and Trp (see Supporting Information). This striking nonlinear effect of poly-**1** enabled the detection of the chirality of Ala with a very small ee less than 0.005% with accuracy and reproducibility (Figure 3B and C). We found a good linear relationship between the ee (from 0.1 to 0.005%) and the ICD values of poly-**1**. These results are qualitatively consistent with a theoretical analysis applied to analogous majority rule effects observed in the polyisocyanates with further experiments required for testing the quantitative correspondence.[22,28] We note that Ala is a typical amino acid frequently found in several meteorites[18] including the Murchison meteorite. Other chiral α-amino acids (0.01% ee of Leu and Ser) and unnatural amino acids such as isovaline (1% ee) (also found in the Murchison meteorite[18c]) can be detected by poly-**1** (see Supporting Information). It is clear from the results reported here that poly-**1** has the potential to detect very small enantiomeric imbalances in a wide variety of amino acids and related molecules of interest in studies of meteorites and elsewhere.

Conclusions

We have developed a novel helical poly(phenylacetylene) that is highly sensitive to amino acid chirality via formation of complexes that exhibit intense ICDs. The advantage of the present helical polymer is not only its high sensitivity to chiral amino acids and amino alcohols on a nanoscale without derivatization, but also its response to all chiral α-amino acids with the same Cotton effect sign if the configurations are the same. The polymer described here may have wide application in the study of nearly racemic amino acids and related molecules of interest, including for extraterrestrial studies.[29]

Experimental Section

Materials. All starting materials were obtained from commercial suppliers and were used as received. 1,4,7,10,13-Pentaoxa-16-azacyclooctadecane (1-aza-18-crown-6) was purchased from Aldrich (Milwaukee, WI). Dry acetonitrile (water content < 0.005 vol %) and dichloromethane (water content < 0.005 vol %) were obtained from Kanto Kagaku (Tokyo, Japan) and Aldrich, respectively. THF was dried over sodium benzophenone ketyl and distilled onto $LiAlH_4$ under nitrogen. Triethylamine was dried over KOH pellet and distilled under nitrogen. These solvents were distilled again under high vacuum just before use. $[(Norbornadiene)\ rhodium\ (I)\ chloride]_2$ ($[Rh(nbd)Cl]_2$) was purchased from Aldrich. L-Alanine and D-alanine (>99.9% ee) were obtained from Peptide Institute, Inc. (Osaka, Japan). DL-Alanine and aqueous perchloric acid solution (60 wt %) were from Kanto Kagaku. L-Leucine, D-leucine, and DL-leucine and tryptophan (purity > 98%) were purchased from Tokyo Kasei (TCI, Tokyo, Japan). Other L-, D-, and DL-amino acids were available from Aldrich. The enantiomeric excesses of these amino acids are not determined by suppliers. (*S*)-Alaninol was purchased from Aldrich, and other amino alcohols used in this study were from TCI. (*S*)-Isovaline (99% purity and ee > 99%) was purchased from Acros Organics (Geel, Belgium). Racemic isovaline was prepared according to the literature procedure.[30]

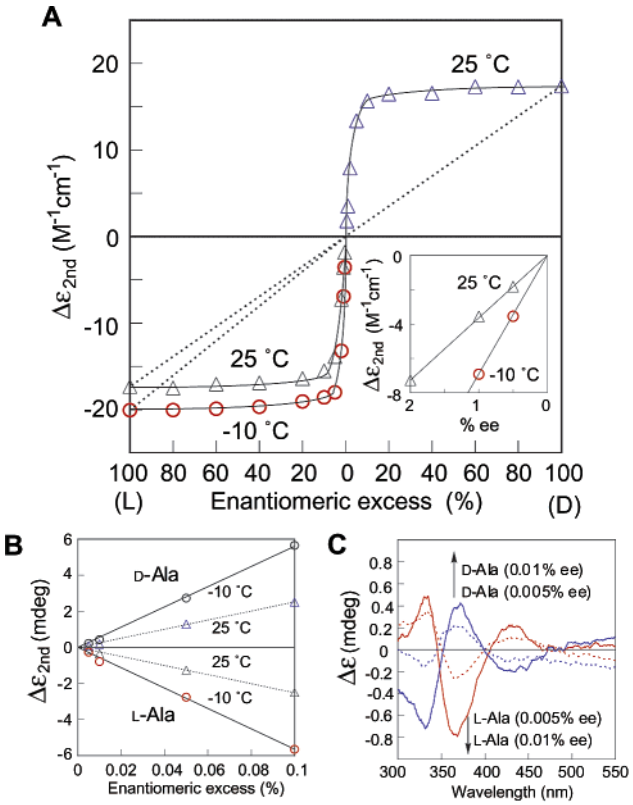


Figure 3. (A) Changes in ICD intensity ($\Delta\epsilon_{2nd}$) of poly-**1** versus the % ee of Ala (10 equiv) during the complexation with poly-**1** in acetonitrile containing 2.8 vol % 1 N $HClO_4$ in water at 25 and −10 °C. The concentration of poly-**1** is 1.0 mg/mL. Inset shows expanded detail of the ICD intensity. (B) Plots between the ICD intensity and 0.1−0.005% ee of Ala at 25 and −10 °C. (C) CD spectra of poly-**1** in the presence of 0.01 and 0.005% ee of L- and D-Ala (10 equiv) at −10 °C.

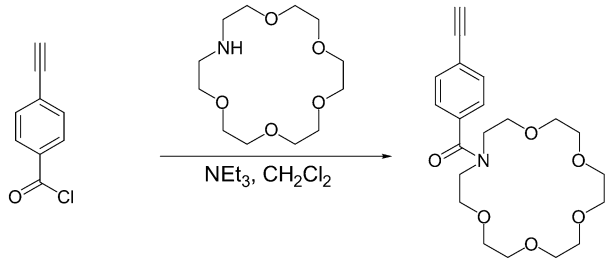
(4-Ethynylbenzoyl)monoaza-18-crown-6 (1). This new compound was synthesized according to Scheme 1. 4-Ethynylbenzoyl chloride was prepared according to the previously reported method.[9] To a mixture of 1-aza-18-crown-6 (1.1 g, 4.2 mmol) in triethylamine (1.6 mL) and dichloromethane (50 mL) was added 4-ethynylbenzoyl chloride (0.97 g, 5.9 mmol) at 0 °C. The mixture was stirred under nitrogen at 25 °C for 7 h. After the solvent was evaporated, the residue was purified by silica gel chromatography with chloroform−methanol (9/1, v/v) as the

(28) (a) Selinger, J. V.; Selinger, R. L. B. *Phys. Rev. Lett.* **1996**, *76*, 58−61. For other references of majority rule in helical polymers, see: (b) Green, M. M.; Garetz, B. A.; Munoz, B.; Chang, H.; Hoke, S.; Cooks, R. G. *J. Am. Chem. Soc.* **1995**, *117*, 4181−4182. (c) Okamoto, Y.; Nishikawa, M.; Nakano, T.; Yashima, E.; Hatada, K. *Macromolecules* **1995**, *28*, 5135−5138. (d) Takei, F.; Onitsuka, K.; Takahashi, S. *Polym. J.* **1999**, *31*, 1029−1032. (e) Tabei, J.; Nomura, R.; Masuda, T. *Macromolecules* **2002**, *35*, 5405−5409.

(29) Welch, C. J.; Lunine, J. I. *Enantiomer* **2001**, *6*, 69−81.

(30) Ogrel, A.; Shvets, V. I.; Kaptein, B.; Broxterman, Q. B.; Raap, J. *Eur. J. Org. Chem.* **2000**, 857−859.

Scheme 1



1

eluent to give 1.5 g of **1** as a white solid (91% yield). Mp (81.4−81.8 °C). IR (KBr, cm^{-1}): 3267 ($\nu_{C\equiv C}$), 1623 ($\nu_{C=O}$), 1138 (ν_{C-O}). ^1H NMR ($CDCl_3$, 500 MHz, TMS): δ 7.51 (d, J = 8.5 Hz, aromatic, 2H), 7.38 (d, J = 8.5 Hz, aromatic, 2H), 3.79−3.56 (m, CH_2, 24H), 3.13 (s, ≡CH, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz, TMS): δ 171.4, 137.1, 132.1, 126.9, 123.0, 83.0, 78.3, 70.8−70.4 (br), 69.6, 69.3, 50.0, 46.0. MS (FAB+): Calcd for $C_{21}H_{30}NO_6$ (M + H), 392. Found: 392. Anal. Calcd for $C_{21}H_{29}NO_6 \cdot {}^1/_3H_2O$: C, 63.46; H, 7.52; N, 3.52. Found: C, 63.40; H, 7.49; N, 3.54.

Polymerization. Polymerization was carried out in a dry glass ampule under a dry nitrogen atmosphere using $[Rh(nbd)Cl]_2$ as a catalyst in a way similar to one previously reported.[6−9] Monomer **1** (0.80 g, 2.1 mmol) was placed in a dry ampule, which was then evacuated on a vacuum line and flushed with dry nitrogen. After this evacuation-flush procedure was repeated three times, a three-way stopcock was attached to the ampule, and dry THF (7.3 mL) and triethylamine (0.3 mL) were added with a syringe. To this was added a solution of $[Rh(nbd)Cl]_2$ in THF (2.0 mL) at 30 °C. The concentrations of the monomer and the rhodium catalyst were 0.2 and 0.002 M, respectively. After 24 h, the resulting poly-**1** was precipitated into a large amount of ether, collected by centrifugation, and dried in vacuo at 50 °C for 2 h (0.76 g, 95% yield). Poly-**1** was soluble in water, methanol, chloroform, acetonitrile, THF, acetone, and toluene. The number average molecular weight (M_n) and molecular weight distribution were 19.7×10^4 and 2.6, respectively, as determined by SEC with polystyrene standards in THF containing 0.1 wt % tetra-*n*-butylammonium bromide as the eluent.

Spectroscopic data of poly-**1**. IR (KBr, cm^{-1}): 1637 ($\nu_{C=O}$), 1107 (ν_{C-O}). ^1H NMR ($CDCl_3$, 60 °C, 300 MHz, TMS): δ 7.11 (s, aromatic, 2H), 6.70 (s, aromatic, 2H), 5.84 (s, =CH, 1H), 3.62 (br, CH_2, 24H). Anal. Calcd for $(C_{21}H_{29}NO_6 \cdot H_2O)_n$: C, 61.60; H, 7.63; N, 3.42. Found: C, 61.86; H, 7.53; N, 3.43.

Instruments. Melting points were measured on a Büchi melting point apparatus and are uncorrected. NMR spectra were taken on a Varian Mercury 300 operating at 300 MHz for ^1H or a Varian VXR-500S spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS) as the internal standard. Fast atom bombardment (FAB) mass spectra were obtained on a JEOL JMS-AX505HA spectrometer (Akishima, Japan). Elemental analyses were performed by the Nagoya University Analytical Laboratory in School of Engineering. SEC measurements were performed with a Jasco PU-980 liquid chromatograph (Jasco, Hachioji, Japan) equipped with a UV (254 nm; Jasco UV-970) detector. A Tosoh (Tokyo, Japan) TSKgel Multipore-H_{XL}-M SEC column (30 cm) was connected, and THF containing 0.1 wt % tetra-*n*-butylammonium bromide was used as the eluent at a flow rate of 0.5 mL/min. The molecular weight calibration curve was obtained with polystyrene standards (Tosoh). IR spectra were recorded using a Jasco Fourier Transform IR-620 spectrophotometer. Absorption and CD spectra were measured in a 1.0-mm quartz cell on a Jasco V-570 spectrophotometer and a Jasco J-820 spectropolarimeter, respectively. The temperature was controlled with a Jasco PTC-423L apparatus (−10−25 °C). Dilute solution viscosities were measured using an Ubbelohde microviscometer at 25 °C in a homemade thermostat.

CD Measurements. Deionized, distilled water and dry acetonitrile were distilled again just before use and stored under nitrogen. The concentration of poly-**1** was calculated on the basis of the monomer units and was 1.0 mg/mL (2.6 mM monomer units) unless otherwise stated. In the complexation of poly-**1** with D- or L-amino acids, stock solutions of poly-**1** (2.0 mg/mL) in acetonitrile and 1 N $HClO_4$ in water were prepared in 5 mL and 50 mL flasks equipped with a stopcock, respectively. Next 0.026 mmol of D- or L-amino acid (2.28 mg of Ala, for example) was placed in a 1 mL flask equipped with a stopcock. Twenty-eight microliters of 1 N $HClO_4$ ($[HClO_4]/[\text{amino acid}] = 1.1$ (mol/mol)) followed by 0.50 mL of the poly-**1** solution were transferred to the 1 mL flask, and the solution was diluted with acetonitrile to keep the poly-**1** concentration at 1.0 mg/mL, and the molar ratio of amino acids to monomer units of poly-**1** was 10. The solution was thoroughly mixed with a vibrator (Iuchi, Osaka, Japan) before measuring the absorption and CD spectra. The same procedure was performed for all of the D- or L-amino acids. The volumetric ratio of water to acetonitrile was held constant at acetonitrile/water = 97.2/2.8 (v/v) for these ICD experiments, but water affects the amino acid-crown ether complexation in aprotic organic solvents,[31] and, therefore, the effect of water on the ICD magnitude of the complex of poly-**1** with L-Ala was investigated on the basis of the CD titrations. We found that the ICD signals of the poly-**1**−L-Ala complex decreased with an increase in the amount of water, so we used pure acetonitrile in the CD titrations of poly-**1** with L-Ala (Figure 2). In the CD titrations in acetonitrile, the L-Ala complexed with $HClO_4$ was prepared according to the reported method.[32] In the CD measurements of poly-**1** with nonracemic Ala (mixtures of L- and D-Ala), a mixture of acetonitrile and aqueous 1 N $HClO_4$ (97.2/2.8, v/v) was used because of difficulty in accurately preparing pure acetonitrile solutions of the Ala−$HClO_4$ complexes with different ee values.

CD Titrations of Poly-1 with L-Ala in Acetonitrile. A stock solution of poly-**1** (2.0 mg/mL) in acetonitrile was prepared in a 10 mL flask equipped with a stopcock. Stock solutions of L-Ala·$HClO_4$ complexes (61.5, 4.56, and 0.41 mM) in acetonitrile were also prepared in 2, 10, and 50 mL flasks, respectively. The 0.50 mL aliquots of the poly-**1** solution were transferred to ten 1 mL flasks equipped with a stopcock. Increasing amounts of the stock solution of the L-Ala·$HClO_4$ complexes were added to the flasks; the molar ratios of L-Ala·$HClO_4$ to poly-**1** were 0.01, 0.02, 0.03 (0.41 mM L-Ala·$HClO_4$ was used), 0.05, 0.1 (4.56 mM L-Ala·$HClO_4$), and 0.5, 1.0, 2.0, 5.0, 10 (61.5 mM L-Ala·$HClO_4$), and the resulting solutions were diluted with acetonitrile to keep the poly-**1** concentrations at 1.0 mg/mL (2.6 mM). The absorption and CD spectra were then taken for each flask to determine the changes in the CD spectra (Figure 2B).

Nonlinear Effects. The changes in the ICD intensity of poly-**1** with respect to the ee of the amino acids (nonlinear effects) were investigated. A typical experimental procedure using Ala is described below. In this experiment, Ala samples were separately prepared for large (1% < ee < 100%) and small (0.001% < ee < 2%) ee values before the CD measurements. The molar ratios of Ala to the monomer units of poly-**1** and $HClO_4$ were held constant at 10 and 0.91, respectively, and the volumetric ratio of water to acetonitrile was held constant at acetonitrile/water = 97.2/2.8 (v/v) unless otherwise stated. The purity of the L- and D-Ala (purchased from Peptide Institute Inc.) was confirmed by high-performance liquid chromatography (HPLC) using a chiral column (Crownpak CR, Daicel, Tokyo, Japan; 25 cm × 0.46 cm (i.d.)) at ambient temperature. Aqueous $HClO_4$ (pH 1.5) was used as the eluent at a flow rate of 0.4 mL/min according to a guidebook supplied from Daicel. In the chromatographic separation of the L- and D-Ala, opposite enantiomers could not be detected, while DL-Ala was completely separated into enantiomers under the same conditions (D- and L-Ala eluted at 3.75 and 4.90 min, respectively), indicating that the enantio-

(31) Zhang, X. X.; Bradshaw, J. S.; Izatt, R. M. *Chem. Rev.* **1997**, *97*, 3313−3361.
(32) Sogah, G. D. Y.; Cram, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 3035−3042.

meric excesses of the L- and D-Ala were greater than 99.9%. Therefore, we assumed that the L- and D-Ala are pure enantiomers and DL-Ala is a 50:50 mixture of L- and D-Ala for preparing the Ala solutions with different ee values. For the preparation of Ala solutions with large and small ee and CD measurements with poly-**1**, see the Supporting Information.

Acknowledgment. This work was partially supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science and the Ministry of Education, Culture, Sports, Science, and Technology, Japan. R.N. expresses thanks for a JSPS Research Fellowship (No. 01428) for Young Scientists.

Supporting Information Available: ^{1}H NMR spectrum of poly-**1**, viscosity measurement results of poly-**1** with or without L-Ala, nonlinear effects between the ICD values for the second Cotton and % ee of Leu and Trp in the complexation with poly-**1**, CD spectra for the complexes of poly-**1** with 0.1, 0.05, 0.01, and 0.005% ee of L- and D-Ala, 0.01% ee of L-Ser and L-Leu, and 5 and 1% ee of L-isovaline, and experimental procedures for the preparation of Ala solutions with large and small enantiomeric excesses (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA028348C