INTRODUCTION

Chirality is an important aspect in living systems. For instance, a pair of enantiomers often exhibits totally different physiological activities depending on the homochirality of the biological molecule. Therefore, rapid and reliable methods for determining the chirality (configuration) and enantiomeric excess (ee) of chiral molecules, particularly chiral drugs, are highly demanded in the pharmaceutical industry (1, 2). Chromatographic enantioseparation by high-performance liquid chromatography (HPLC) has mostly been used for this purpose because it allows the precise determination of ee for various nonracemic compounds produced by the state-of-the-art asymmetric catalysis and traditional optical resolution of racemates (3, 4).

Another interesting class of techniques is based on enantioselective molecular probes/sensors (5–12). These methods provide only a rough estimation of the ee values, not as accurate as those determined by chiral HPLC. This is mostly because of the linear relationship between the full range of ee values and output signals, leading to unignorable deviations (errors) (Fig. 1A) (13, 14). Analyn and co-workers developed an elegant method for the accurate determination of ee values of various chiral molecules in the high–ee region (15, 16). The method was based on the use of covalent and noncovalent helical polymer systems that exhibited nonlinear circular dichroism (CD)–ee relationships (10, 17–19). Such a nonlinear chiral response to ee has a substantial advantage over the linear chiral response, because chiral signals, particularly in the narrow, high, and/or low–ee regions of interest, can be remarkably amplified, thereby allowing the quantification of ee values with high accuracy (Fig. 1A).

Among the promising alternatives is the direct colorimetric discrimination between enantiomers and the simultaneous determination of ee values of the target chiral compounds, based on the differences in absorbance and fluorescence emission, thereby enabling the naked-eye detection of chirality. This is, however, challenging, and successful examples of these systems are limited due to the distinct conformational scaffold of low–molecular weight chiral receptors, which exhibit a linear response (20–27).

We recently reported that nonracemic amines in water induced the folding of π-conjugated fluorescent poly(diphenylacetylene) (PDPA) bearing carboxy pendants (poly-1-H) into a one-handed helix upon thermal annealing. The right (P)– or left (M)–handed helical conformation induced in poly-1-H by nonracemic amines could be retained, namely, “memorized,” after complete removal of the chiral amines, resulting in the formation of the one-handed helical P- or M-h-poly-1-H with static helicity memory, respectively (Fig. 1B) (28). As part of our ongoing program to develop h-poly-1-H–based advanced chiral materials for separating enantiomers (29), we serendipitously found that M-h-poly-1-Hs modified with (S)- and (R)-1-phenylethylamine (S- and R-2a), namely, M-h-poly-1-S2a and M-h-poly-1-R2a, respectively, showed completely different colors in specific solvents.

Here, we report an unprecedented helical polymer–based versatile color indicator that allows not only the assignment of the absolute configuration of chiral amines but also the quantitative determination of their ee values in the full range of ee. This was achieved by digital photography by converting to the RGB (red, green, and blue) values (Fig. 1, B and C). The method was as accurate as chiral HPLC. The present helical polymer–based color change relies on the nonlinear response of a change in the tunable helical pitch of the π-conjugated polymer backbone like a helical spring, which results from the formation or disruption (switching on/off) of the intramolecular hydrogen (H)–bonding networks among the pendant amides in specific polar solvent mixtures. This allows the rapid, on-site monitoring of the chirality of nonracemic amines and the simultaneous quantitative determination of their ee values. A rapid and simple system for the precise determination of ee of chiral amines, which are important components or precursors for pharmaceuticals and pesticides, would be particularly useful (4, 7, 12–14).

RESULTS AND DISCUSSION

The one-handed helical M-h-poly-1-H and P-h-poly-1-H with static helicity memories were prepared from optically inactive poly-1-H
The carboxy groups be quantified by digital photography by converting to RGB values.

The nonlinear response of a change in the helical pitch of the chromophoric polymer backbone triggered by the on/off switch—solvent polarity–regulated formation of intramolecular H-bonding network among the amide pendants. The ee can also be visually discriminated by its fluorescence (Fig. 2C). The absorption and CD spectra of M-h-poly-1-R2a, M-h-poly-1-R2a in THF-acetone (9/1, v/v) were determined to be 30.9% and 8.0%, respectively, using quinine sulfate in aqueous sulfuric acid (0.1 M) as a standard material (30). Hence, the chirality of 2a can also be visually discriminated by its fluorescence (Fig. 2C).

As expected, the spectral behaviors of M-h-poly-1-S2a and P-h-poly-1-R2a prepared from the opposite right-handed helical P-h-poly-1-H were totally opposite to those of M-h-poly-1-R2a and M-h-poly-1-S2a, respectively, and mirror-image CD spectra were obtained in THF-acetone (9/1, v/v) (fig. S10). In addition, the colorimetric and spectral differences between M-h-poly-1-S2a and M-h-poly-1-R2a were highly dependent on the hse of h-poly-1-H and decreased with decreasing hse values of h-poly-1-H before its modification (fig. S11). The reactions of h-poly-1-H with R- and S-2a using DMT-MM proceeded very quickly; the reaction reached completion within ~3 min, as confirmed by infrared (IR) spectroscopy (fig. S12). After diluting the reaction mixture with chloroform, we could visually discriminate the absolute configuration of 2a, without isolating the modified polymers (movie S1). Thus, a practically useful dual-mode, on-site chiral sensor (visual differences in solution color and fluorescence emission) could be developed.

It was possible to develop a similar assay for the naked-eye detection of chirality for various chiral amines (2b to 2j), amino alcohols (2k to 2s), and amino acid esters (2t1 to 2t10) upon their reaction with M-h-poly-1-H (Fig. 3A), followed by solvent (fig. S13) and temperature (fig. S14) optimization. For simple chiral amine 2d, amino alcohols 2k to 2n, and amino acid methyl esters 2t1 to 2t4, naked-eye detection was possible at a low temperature (~60° C or ~50° C). The colorimetric response from the amino acid esters notably improved by introducing bulkier ester groups, particularly, the tert-butyl and benzyl ester groups, allowing the naked-eye detection of the enantiomers at 25°C (2t7 to 2t10) (Fig. 3A and fig. S13). All the tested primary amines (2a to 2j), amino alcohols (2k to 2s), and amino acid esters (2t1 to 2t10) with the same configuration as R2a, S2k, and L2h1, respectively, exhibited more prominent color changes than their corresponding enantiomeric counterparts after functionalization with M-h-poly-1-H except for 2q and 2r, of which the (R)-enantiomers exhibited a more prominent color change because of difference in the priority sequences (see fig. S13B). This allowed the quick assignment of the chiral amine configurations by simple visible inspection. Enantiomers of representative drug-related compounds, such as amphetamine (2j)—a stimulant drug and a
metabolite of other stimulant drugs, and phenylpropylamine (nor-pseudoephedrine and norephedrine) (2s), could also be visually discriminated (Fig. 3A and fig. S15). Their chiral discrimination is important because it is useful to distinguish the source of the drugs (2).

Four diastereomers of 2u with two stereogenic centers could also be discriminated by the naked eye and fluorescence emission upon irradiation at 365 nm (Fig. 3B and fig. S16). In sharp contrast, M-h-poly-1-S2aMe and M-h-poly-1-R2aMe composed of secondary chiral amines prepared from N-methylated 2a (S- and R-2aMe) exhibited almost identical absorption and CD spectra (fig. S15A), suggesting that cooperative intramolecular H-bonding between the neighboring amide pendants along the helical backbone is crucial in the colorimetric chiral discrimination by this system and chiral secondary amines cannot be applied to this method.

Direct evidence for the difference between the intramolecular H-bonding among the adjacent amide pendants in red-colored
M-h-poly-1-R2a and yellow-colored M-h-poly-1-S2a in THF-acetone (9/1, v/v) was obtained from amide hydrogen-deuteration (H/D) exchange experiments (Fig. 4B and fig. S21). The half-lives of the H/D exchange for the amide protons of M-h-poly-1-R2a and M-h-poly-1-S2a, as estimated from the Arrhenius analysis, are 19 and 2 hours, respectively, indicating that the intramolecular H-bonding in red-colored M-h-poly-1-R2a was much stronger than in yellow-colored M-h-poly-1-S2a; this was also supported by the IR measurements (fig. S22).

Molecular mechanics calculations revealed that cis-cisoidal M-h-poly-1-R2a can form regular intramolecular H-bonds between the neighboring amide pendants, whereas cis-cisoidal M-h-poly-1-S2a can only partially form these intramolecular H-bonds (fig. 4A and fig. S23). Moreover, this regular intramolecular H-bonding is impossible in cis-transoidal M-h-poly-1-R2a and M-h-poly-1-S2a (Fig. 4A). Therefore, the behaviors of M-h-poly-1-R2a and M-h-poly-1-S2a can be ascribed to a spring-like helical conformational change in the contracted cis-cisoidal helical conformation (red-colored) and stretched cis-transoidal conformation (yellow-colored), respectively (31); the color change in the former is triggered by the formation of regular intramolecular H-bonding networks among the amide pendants in specific polar solvents, thus acting as an on/off switch.

X-ray diffraction (XRD) patterns of red-colored M-h-poly-1-R2a and yellow-colored M-h-poly-1-R2a and M-h-poly-1-S2a films showed a strong reflection at 17.8 Å, which can be indexed to (100) reflections, suggesting a columnar pseudohexagonal packing (Fig. 4C and fig. S24) (31). The red-colored M-h-poly-1-R2a film showed a weak but apparent reflection at 28.5°, assigned to the π-π stacking of the pendant phenyl rings (3.1 Å). In contrast, the yellow-colored films showed no observable reflection in the same region. These results suggested that red-colored M-h-poly-1-R2a forms π-π stacking of the pendant phenyl rings by adopting the contracted cis-cisoidal conformation through the formation of intramolecular H-bonds between the neighboring amide groups (Fig. 4A, right bottom); this π-π stacking is not available in the yellow-colored, stretched cis-transoidal M-h-poly-1-R2a and M-h-poly-1-S2a.

These results indicate that the degree of color change (difference) between the diastereomeric amide-bound one-handed helical polymers resulting from the formation or disruption (switching on/off)
of the intramolecular H-bonding networks among the pendant amides is most likely determined by a delicate balance of the polarity and the intermolecular H-bonding ability (solvation) of the solvents with the chiral amide residues, temperature, and steric effect of the hydrophobic or hydrophilic substituents on the stereogenic center of the amide pendant derived from the enantiomers of primary amines. When one-handed helical M-h-poly-1-H was modified with chiral amines and amino alcohols bearing a bulky hydrophobic substituent [e.g., phenyl (2a, 2f to 2h, 2p, and 2r), naphthyl (2b and 2i), cyclohexyl (2c), and benzyl (2j and 2o)] over the other small substituents on the stereogenic center, the naked-eye detection of the enantiomers of 2d and 2k required low-temperature measurements in less polar chloroform as anticipated (Fig. 3A) because of easy access of solvent molecules to the amide groups along with insufficient chiral steric effect, which will prevent the formation of the intramolecular H-bonds between the neighboring amide pendants at 25°C. As for the amino acid methyl esters (2t1 to 2t4), the methyl ester residue is bulky but hydrophilic so that the amide residues are more easily solvated, although 2t5 as the ethyl (fig. S14). Hence, introducing bulkier hydrophobic ester groups, such as methyl isobutyl ketone (MIK), DMF, dimethylacetamide (DMA), and dimethyl sulfoxide (DMSO) in THF (Fig. S28); polar solvents with higher dielectric constants (ε; table S1) showed a larger shift upon the addition of a small amount of the polar solvent (32). Similarly, naked-eye detection of the configuration and ee values of other chiral amines (2b, 2c, 2p, and 2t7) is possible after simple and quick functionalization of the amines with M-h-poly-1, followed by their dissolution in appropriate solvent mixtures (figs. S29 and S30).

Taking advantage of the unique ee-dependent color changes of h-poly-1-2a regulated by the solvent polarity, more precise determination of the ee of 2a, based on the color change, was conducted in various narrow ee regions. For example, M-h-poly-1-RX2a [X (% ee) = 0, 6, 12] could be clearly distinguished by naked eyes using a THF-acetone mixed solvent (65/35, v/v; Fig. 5D and fig. S31). Moreover, M-h-poly-1-RX2a [X (% ee) = 90 to 100, in steps of 2] prepared by the reaction of M-h-poly-1-H with the corresponding chiral amines RX2a (table S3) showed substantial changes in the absorption intensity at 551 nm and apparent visible color changes with respect to the ee values of 2a in THF-DMF (79/21, v/v). This allowed quantification of the difference in the ee values with high accuracy (Fig. 5, E and F). Naturally, when P-h-poly-1-H, with opposite helicity memory, was modified with SX2a [X (% ee) = 90 to 100, in steps of 2], the P-h-poly-1-SX2a enantiomers (table S3) displayed absorption spectral changes and color changes (fig. S32) similar to those of M-h-poly-1-RX2a. It is also to be noted that optically active M- and P-h-poly-1-H compounds reacted with nonracemic 2a in a nonselective way (table S4). The ee difference between R90 and R100 of 2a could also be visually discriminated by their fluorescence (fig. S33).

The ee values of “unknown” 2a samples, ranging from R90 to R100, were estimated from their absorption spectra based on the calibration curve. The values were very close to the ee values determined by chiral HPLC, and the errors were relatively low (fig. S34 and table S5) (16, 33). Furthermore, colorimetric determination of ee without using any spectroscopic instruments was possible by taking photographs of the solutions and converting them to RGB values (fig. S34D). Hence, we could estimate the ee values of RX2a (X ≥ 90) with high accuracy from the plots of the intensities of the G (green) component (Fig. 5G and table S5).

To investigate whether this system can detect an extremely small difference in the ee in a sample with very high ee of 2a (≥98), M-h-poly-1-RX2a [X (% ee) = 98 to 100, in steps of 0.5] was also prepared (table S3). Apparent absorption spectral changes were observed for M-h-poly-1-RX2a (X ≥ 98) in THF-DMF (78/22, v/v) (Fig. 5H); the plots of their absorbance at 545 nm were linear with respect to the ee values of 2a (Fig. 5I). The P-h-poly-1-SX2a enantiomers with the opposite helicity memory (table S3) showed identical absorption spectral changes (fig. S35). These results demonstrated that using this unique colorimetric sensor, a difference in the ee values as small as 0.5% ee, even in a sample with a very high ee (≥98), could be detected by acquiring the absorption spectra.
We envisage that the pendant carboxy groups of h-poly-1-H can be replaced with various other functional groups while maintaining its macromolecular helicity memory. This should be applicable to the on-site, naked-eye determination of ee of various functional molecules and biologically relevant compounds.

MATERIALS AND METHODS

Materials
Tungsten(VI) chloride (WCl₆) and (trimethylsilyl)diazomethane (TMSD; 2 M in diethyl ether) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetraphenyltin (Ph₄ Sn) and L-(−)- and D-(+) mandelic acids were obtained from Tokyo Chemical Industry (TCI; Tokyo, Japan). Potassium hydroxide (KOH) and 2-methoxy-4-nitrobenzoic acid were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan). Optically active amines (2a to 2i, 2k to 2r, 2t₁ to 2t₁₀, 2u, and 2a(Me)) (Fig. 3 and fig. S3) were obtained from Sigma-Aldrich, FUJIFILM Wako Pure Chemical, TCI, Nacalai Tesque (Kyoto, Japan), and Watanabe Chemical Industries (Hiroshima, Japan) and used as received except for 2a (see the Supplementary Materials). (1S,2R)- and (1R,2S)-norephedrine (1S₂R⁻ and 1R₂S⁻2s) were purchased from FUJIFILM Wako Pure Chemical. (S) - and (R)-amphetamine (S- and R-2j) (34) and (1S,2S)- and (1R,2R)-norpseudoephedrine (1S₂S⁻ and 1R₂R⁻2s) (35) were synthesized from the corresponding norephedrine according to the literature method. Anhydrous THF, toluene, DMSO, and DMF were purchased from Kanto Kagaku (Tokyo, Japan), and these solvents were stored under nitrogen. Dehydrated acetone and MIK were obtained from FUJIFILM Wako Pure Chemical.
Pure Chemical. DMT-MM was prepared according to the reported method (36). Poly-1-H was prepared according to the previously reported method (see the Supplementary Materials) (28, 29).

**Instruments**

Nuclear magnetic resonance (NMR) spectra were taken on a JNM-ECA 500 spectrometer (500 MHz for $^1$H, 125 MHz for $^{13}$C) (JEOL, Akishima, Japan) or a Bruker Avance 400 spectrometer (400 MHz for $^1$H, 100 MHz for $^{13}$C) (Bruker, MA, USA) in CDCl₃, DMSO-$d_6$, DMF-$d_7$, CD$_3$OD, THF-$d_8$, THF-$d_8$-acetone-$d_6$ (9/1, v/v), and THF-$d_8$-acetone-$d_6$ (9/1, v/v). IR spectra were recorded with a JASCO Fourier Transform IR-460 spectrophotometer (JASCO, Tokyo, Japan). Absorption and CD spectra were measured in a 1.0-mm, 10-mm, or 0.1-mm quartz cell on a JASCO V-650 spectrophotometer and a JASCO J-725 spectropolarimeter, respectively. The temperature was controlled with a JASCO PTC-348W1 apparatus. The concentration of polymers was calculated on the basis of the monomer units. Size exclusion chromatography (SEC) measurements were performed with a JASCO PU-2080 liquid chromatograph equipped with an ultraviolet (UV)–vis (JASCO UV-970) detector at 40°C using a Shodex (Tokyo, Japan) GF-805L SEC column. The temperature was controlled with a JASCO CO-1560 column oven. THF was used as the eluent at a flow rate of 1.0 ml/min. The molar mass calibration curves were obtained with polystyrene standards (Tosoh, Tokyo, Japan). Photoluminescence spectra were measured on a JASCO FP-8500 spectrophotometer. The temperature was controlled with a JASCO ETC-815 apparatus. XRD measurements were performed on Nano Viewer (RA-MICROT7HM) (Rigaku Corporation, Tokyo, Japan). Ee values of chiral amines ($2a$ to $2c$, $2p$, and $2q$) were determined by HPLC equipped with a photodiode array detector (JASCO MD-4010) and a CD detector (JASCO CD-4095) at room temperature (see the Supplementary Materials). A chiral column [CHIRALPAK IG-3; 250 × 4.6 mm inner diameter (i.d.); Daicel, Osaka, Japan] was connected, and n-hexane–dichloromethane (1/1, v/v) was used as the eluent at a flow rate of 0.5 ml/min. A chiral column [CROWNPAK CR-1 (–); 250 × 4.6 mm i.d.; Daicel] was also used for ee determination of $2t$–HCl. pH 1.5 HClO$_4$ aq./CH$_3$CN (4/1, v/v) was used as the eluent at a flow rate of 0.4 ml/min. Elemental analyses were performed by the Research Institute for Instrumental Analysis of Advanced Science Research Center, Kanazawa University, Kanazawa, Japan.

**Synthesis**

**General procedure for the modification of M- and P-h-poly-1-Hs with chiral amines**

The reactions of $M$-h-poly-1-H or $P$-h-poly-1-H with chiral amines ($2a$ to $2u$ and $2a^{Me}$) (Fig. 3 and fig. S3) were carried out with DMT-MM as the condensing reagent, as shown in fig. S4. A typical experimental procedure for the reaction of $M$-h-poly-1-H with chiral amine $2a$ is described below.

S-$2a$ (95.7 μl, 0.75 mmol) and DMT-MM (209 mg, 0.75 mmol) were added to a solution of $M$-h-poly-1-H (50.4 mg, 0.19 mmol) in a DMSO-water mixed solvent (5/1, v/v) (10 ml), and the resulting mixture was stirred at room temperature for 4 hours. The resulting polymer was precipitated into a large amount of methanol-water mixture (1/1, v/v), collected by centrifugation, washed with methanol-water mixture (1/1, v/v), and then dried in vacuo at room temperature overnight to yield $M$-h-poly-1-$2a$ (85.0 mg, 0.18 mmol, 95% yield). The side groups of $M$-h-poly-1-H were completely modified with $S$-$2a$ as confirmed by its $^1$H NMR and elemental analysis.

In the same way, modifications of $M$-h-poly-1-H with $R$-$2a$ and other various $R$ and $S$ amines ($2b$ to $2u$ and $2a^{Me}$) were performed to afford the corresponding $M$-h-poly-1-$R_{2a}$s and $M$-h-poly-1-$S_{2a}$s. The complete modifications of the side groups were confirmed by $^1$H NMR, IR, and elemental analyses (see the Supplementary Materials and also table S6 and fig. S12).

**Procedure for the absorption spectral measurements of $M$-h-poly-1-$R_{2a}$ and $P$-h-poly-1-$S_{2a}$ ($X > 90\%$ ee)**

Stock solutions of $M$-h-poly-1-$R_{2a}$ (7.5 mM) ($X = 90, 92, 94, 96, 98$, and 100% ee) in THF were prepared in 2-ml flasks equipped with a stopcock. A 200-μl aliquot of each $M$-h-poly-1-$R_{2a}$ stock solution was transferred to three vials using a Hamilton microsyringe. THF was completely removed under a high vacuum to give three vials containing 1.5 μmol of $M$-h-poly-1-$R_{2a}$. A mixed solvent THF-DMF (79/21, v/v) was prepared by mixing THF (140.30 g) and DMF (39.69 g) in a bottle with a Teflon screw cap. A 3-ml aliquot of the mixed solvent was added to the vials to keep the $M$-h-poly-1-$R_{2a}$ concentrations at 0.5 mM. The absorption spectra were taken at 25°C using a 10-mm quartz cell for each vial, and the isosbestic point was observed at 456 nm. The concentration of the polymers was corrected using the ε (molar absorptivity) value ($\varepsilon_{456} = 2.0 \times 10^5 \text{M}^{-1} \cdot \text{cm}^{-1}$). The average absorption intensity at 551 nm of each $M$-h-poly-1-$R_{2a}$ sample was determined on the basis of the results of three vials. The average absorption intensities at 551 nm were plotted versus the % ee values of the samples determined by the chiral HPLC analysis (tables S2 and S3 and fig. S32C). This relationship was used as the calibration curve for ee determination of blind unknown $M$-h-poly-1-$R_{2a}$s ($X > 90$% ee) samples using absorption intensities at 551 nm (fig. S34B) (see below). The same procedure was used for the absorption spectral measurements of $P$-h-poly-1-$S_{2a}$ ($X = 90, 92, 94, 96, 98$, and 100% ee).

The absorption spectral measurements of $M$-h-poly-1-$R_{2a}$ and $P$-h-poly-1-$S_{2a}$ ($X = 98.0, 98.5, 99.0, 99.5$, and 100% ee) were carried out in a similar way by using a mixed solvent THF-DMF (78/22, v/v), which was prepared by mixing THF (138.53 g) and DMF (41.58 g) in a bottle with a Teflon screw cap. The absorption spectra were taken at 25°C using a 10-mm quartz cell for each vial, and the isosbestic point was observed at 456 nm. The concentration of the polymers was corrected using the ε (molar absorptivity) value ($\varepsilon_{456} = 2.0 \times 10^5 \text{M}^{-1} \cdot \text{cm}^{-1}$). The average absorption intensity at 545 nm of each $M$-h-poly-1-$R_{2a}$ sample was determined on the basis of the results of three vials. The average absorption intensities at 545 nm were plotted versus the % ee values of the samples determined by the chiral HPLC analysis (tables S2 and S3 and fig. S35D). This relationship was used as the calibration curve for ee determination of blind unknown $M$-h-poly-1-$R_{2a}$ samples. A mixed solvent THF-DMF (79/21, v/v) was prepared by mixing THF (140.30 g) and DMF (39.69 g) in a bottle with a Teflon screw cap. A 3-ml aliquot of the mixed solvent was added to the vials to keep the $M$-h-poly-1-$R_{2a}$ concentrations at 0.5 mM. The absorption spectra were taken at 25°C using a 10-mm quartz cell for each vial, and the isosbestic point was observed at 456 nm. The concentration of the polymers was corrected using the ε (molar absorptivity) value ($\varepsilon_{456} = 2.0 \times 10^5 \text{M}^{-1} \cdot \text{cm}^{-1}$). The average absorption intensity at 545 nm of each $M$-h-poly-1-$R_{2a}$ sample was determined on the basis of the results of three vials. The average absorption intensities at 545 nm were plotted versus the % ee values of the samples determined by the chiral HPLC analysis (tables S2 and S3 and fig. S35D). This relationship was used as the calibration curve for ee determination of blind unknown $M$-h-poly-1-$R_{2a}$ samples. A mixed solvent THF-DMF (79/21, v/v) was prepared by mixing THF (140.30 g) and DMF (39.69 g) in a bottle with a Teflon screw cap. A 3-ml aliquot of the mixed solvent was added to the vials to keep the $M$-h-poly-1-$R_{2a}$ concentrations at 0.5 mM. The absorption spectra were taken at 25°C using a 10-mm quartz cell for each vial, and the isosbestic point was observed at 456 nm. The concentration of the polymers was corrected using the ε (molar absorptivity) value ($\varepsilon_{456} = 2.0 \times 10^5 \text{M}^{-1} \cdot \text{cm}^{-1}$). The average absorption intensity at 545 nm of each $M$-h-poly-1-$R_{2a}$ sample was determined on the basis of the results of three vials. The average absorption intensities at 545 nm were plotted versus the % ee values of the samples determined by the chiral HPLC analysis (tables S2 and S3 and fig. S35D). This relationship was used as the calibration curve for ee determination of blind unknown $M$-h-poly-1-$R_{2a}$ samples.
was performed in the same way as described above [THF-water mixed solvent (4/1, v/v) was used instead of DMSO-water mixed solvent] to afford M-h-poly-1-R2aA and M-h-poly-1-R2aB.

Procedure of the determination of the ee values of blind unknown Rx-2a samples after converting to M-h-poly-1-Rx2a based on their absorption spectra. Stock solutions of M-h-poly-1-R2aA and M-h-poly-1-R2aB (7.5 mM) in THF were prepared in 2-ml flasks equipped with a stopcock. A 200-µl aliquot of each M-h-poly-1-R2aA and M-h-poly-1-R2aB stock solution was transferred to three vials using a Hamilton microsyringe. THF was completely removed under a high vacuum to give three vials containing 1.5 mol of M-h-poly-1-R2aA and M-h-poly-1-R2aB. A mixed solvent THF-DMF (79/21, v/v) was prepared by mixing THF (140.30 g) and DMF (39.69 g) in a bottle with a Teflon screw cap. A 3-ml aliquot of the mixed solvent was added to the vials to keep the M-h-poly-1-R2aA and M-h-poly-1-R2aB concentrations at 0.5 mM. The absorption spectra were taken at 25°C using a 10-mm quartz cell for each vial, and the isosbestic point was observed at 456 nm (fig. S34A). The calibration curve was obtained from the relationship between the absorption intensities at 551 nm and the ee % values of the samples by taking their photographs. Ten spots (fig. S34B and table S5).

Color Meter (Apple Inc.), which is a built-in Mac utility. Ten spots were taken on July 5, 2021 http://advances.sciencemag.org/Downloaded from

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/27/eabg5381/DC1

**REFERENCES AND NOTES**


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Helical springs as a color indicator for determining chirality and enantiomeric excess
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