Supporting information

Enantioselective resolution of two model amino acids using inherently chiral oligomer films with uncorrelated structures

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1) Materials and methods

Starting materials Racemic asparagine monohydrate (*DL*-ASN·H_2O) was purchased from TCI with a purity of >98%. Enantiopure 2,2´-bis(2,2´-bithiophene-5-yl)-3,3´-bithianaphthene (BT_2-T_4) monomers (595 g/mol MW) were synthesized as a racemate following the procedures described by *Sannicolò et.al*, and subsequently separated into enantiopure antipodes by chiral HPLC coupled with a CD detector.[1] The properties of BT_2T_4 have been studied and reported in more detail in previous publications.[2-4] Milli-Q water (resistivity = 18.2 MΩ•cm) was used as solvent in all experiments.

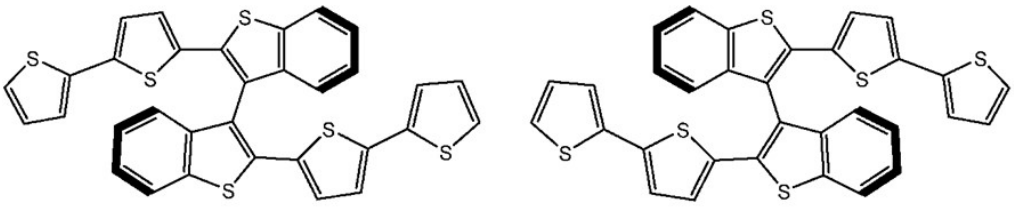
Oligomerization of oligo-(*R*)-/(*S*)-BT_2-T_4 films Gold coated glass substrates (1x1 cm^2) were cleaned with acetone before using them as working electrodes for electrooligomerization. A 0.5 mM monomer solution (BT_2T_4) was prepared with 0.1 M $TBAPF_6$ in acetonitrile as supporting electrolyte. Afterwards the electrooligomerization of the corresponding enantiopure monomers was carried out by potentiodynamic scanning at a scan rate of 0.1 V/s in a potential range from 0 to 1.25 V vs Ag/AgCl (KCl saturated) for 20 cycles.[4] In order to obtain doped films, the oligomerization procedure was carried out under the same conditions, but stopping the oligomerization at 1.25 V as the final potential. In this case the oligomer is in its doped state (positively charged) exhibiting an orange/green colour, whereas it is yellowish in its undoped state. The oligomerization of achiral oligo-templates was carried out using a monomer solution containing 0.25 mM of each *R*- and *S*-BT_2T_4 with 0.1 M $TBAPF_6$ in acetonitrile as supporting electrolyte. The rest of the protocol was the same as mentioned above.

Crystallization Supersaturated solutions of *DL*-ASN·H_2O were prepared by dissolving 0.076 g of *DL*-ASN·H_2O in 1 ml of water (the solubility at 30 °C)[5] at 40 °C to ensure that no crystalline material remains in the solution. The oligo-(*R*) or oligo-(*S*)-BT_2T_4 modified substrates were placed, separately, vertically in a beaker containing the supersaturated solution of *DL*-ASN located in the center of a double-walled reactor. The temperature was controlled by using a thermostat (Julabo temperature control apparatus model F32). The temperature program started at 21 °C and the temperature was continuously decreased to 17 °C with a 0.5 °C/h cooling rate. Then, the temperature was held at 17 °C until the crystallization occurred. After crystallization, the template was removed from the solution and immediately washed with cold milli-Q water. Then, the crystals were collected from the surface of the oligomer template and analyzed by HPLC, using the method described below. The crystallinity of the obtained ASN single crystals was checked by using optical microscopy (see Figure S3).

Contact angle measurement The average wettability (water contact angle) of the oligomer films was measured using a contact angle goniometer (Ossila, England) by placing a drop of milli-Q water (2 µL) on three different locations along the oligomer surface. The average value was reported as the measured contact angle.

High-Performance Liquid Chromatography (HPLC) A Shimadzu Prominence-i LC-2030C 3D HPLC equipped with a UV-vis lamp and a detector was used to determine the enantiomeric excess of crystalline ASN·H_2O. A reversed-phase HPLC column, Shodex ORpak CRX-853 (8mm x 5mm x 6m) was used to analyze the enantiomer content of ASN, using 0.5 ml/min of 1.5 mM $CuSO_4$ in aqueous solution as a mobile phase at 25 °C. For Glu analysis, an Astec CHIROBIOTIC T was used with 1 ml/min of water: methanol: formic acid (20:80:0.02) as a mobile phase at 23 °C.

2) Chemical structure of the inherent chiral monomer



(S)-BT_2T_4 *(R)*-BT_2T_4

Figure S1 Enantiomers of 2,2´-bis(2,2´-bithiophene-5-yl)-3,3´-bithianaphthene (BT_2T_4)

3) Cross-section of an oligo-(*S*)-BT_2T_4 film with EDS analysis

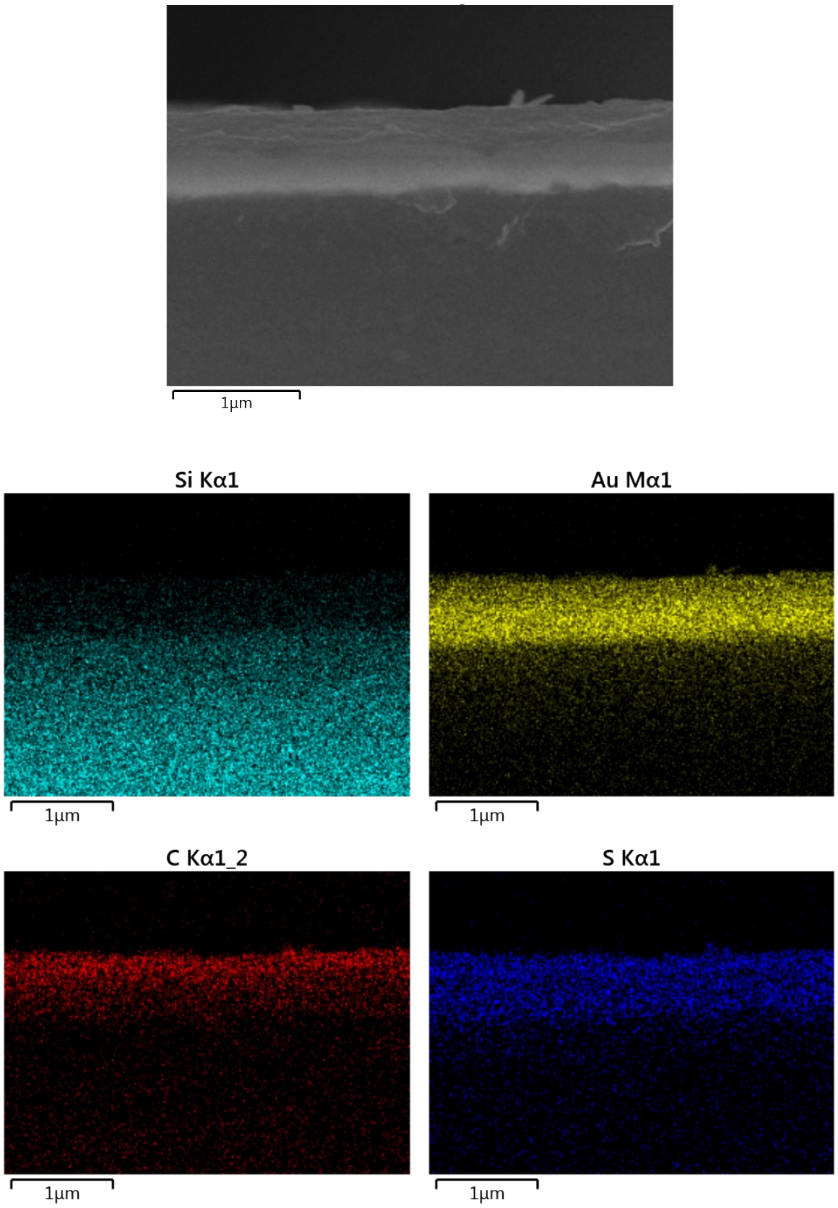


Figure S2 The cross-section image and EDS elemental analysis of an oligo-(*S*)-BT_2T_4 film.

4) Characterization of oligomer films

Table S1 Wettability of oligomer films

Samples	Average contact angle	Standard deviation
unmodified gold	107.95	1.87
Oligo-(*R*)-BT_2T_4 undoped template no.1	112.90	1.73
oligo-(*R*)-BT_2T_4 undoped template no.2	115.48	1.27
oligo-(*R*)-BT_2T_4 doped template no.1	58.85	4.08
oligo-(*R*)-BT_2T_4 doped template no.2	59.91	1.51

5) Chiral resolution of *DL*-ASN·H_2O using fresh oligo-(*R*)-/(*S*)-BT_2T_4.

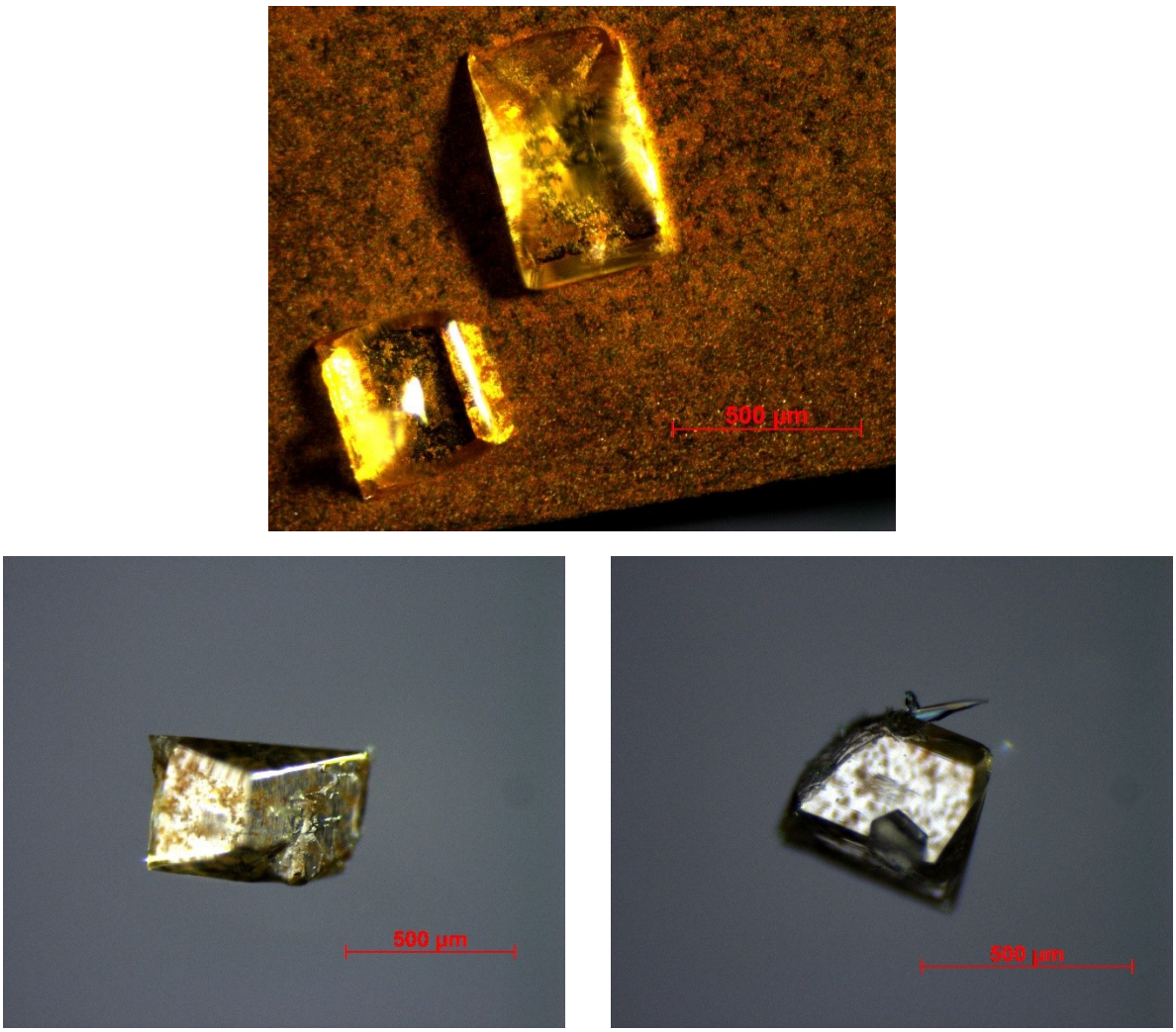
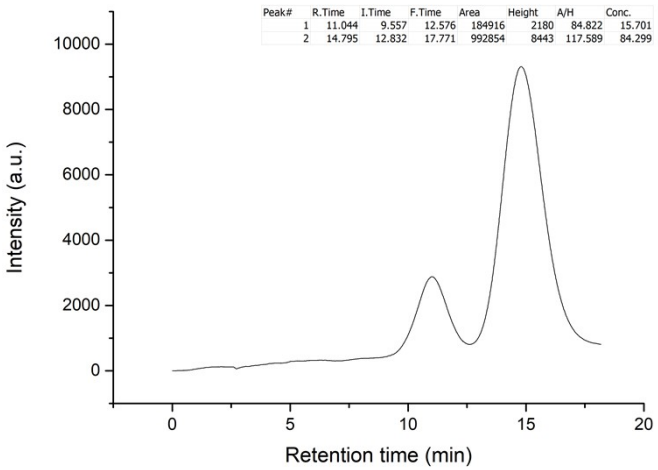
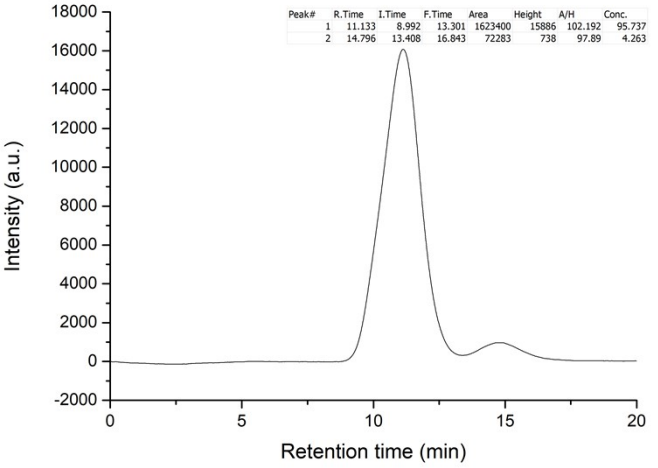


Figure S3 ASN crystals obtained from the chiral resolution experiment using the oligo-(*S*)-BT_2T_4 film**.**

a)



b)



c)

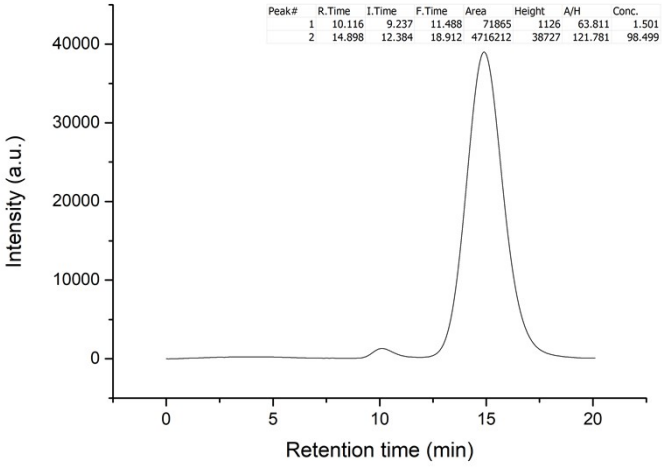


Figure S4 Examples of HPLC chromatograms (detection at 254 nm) presenting the % *ee* from the chiral resolution of *DL*-ASN using a) undoped oligo-(*S*)-BT_2T_4 b) undoped oligo-(*R*)-BT_2T_4 c) doped oligo-(*S*)-BT_2T_4.

6) Polymer film cleaning for recycling experiments

6.1) HPLC

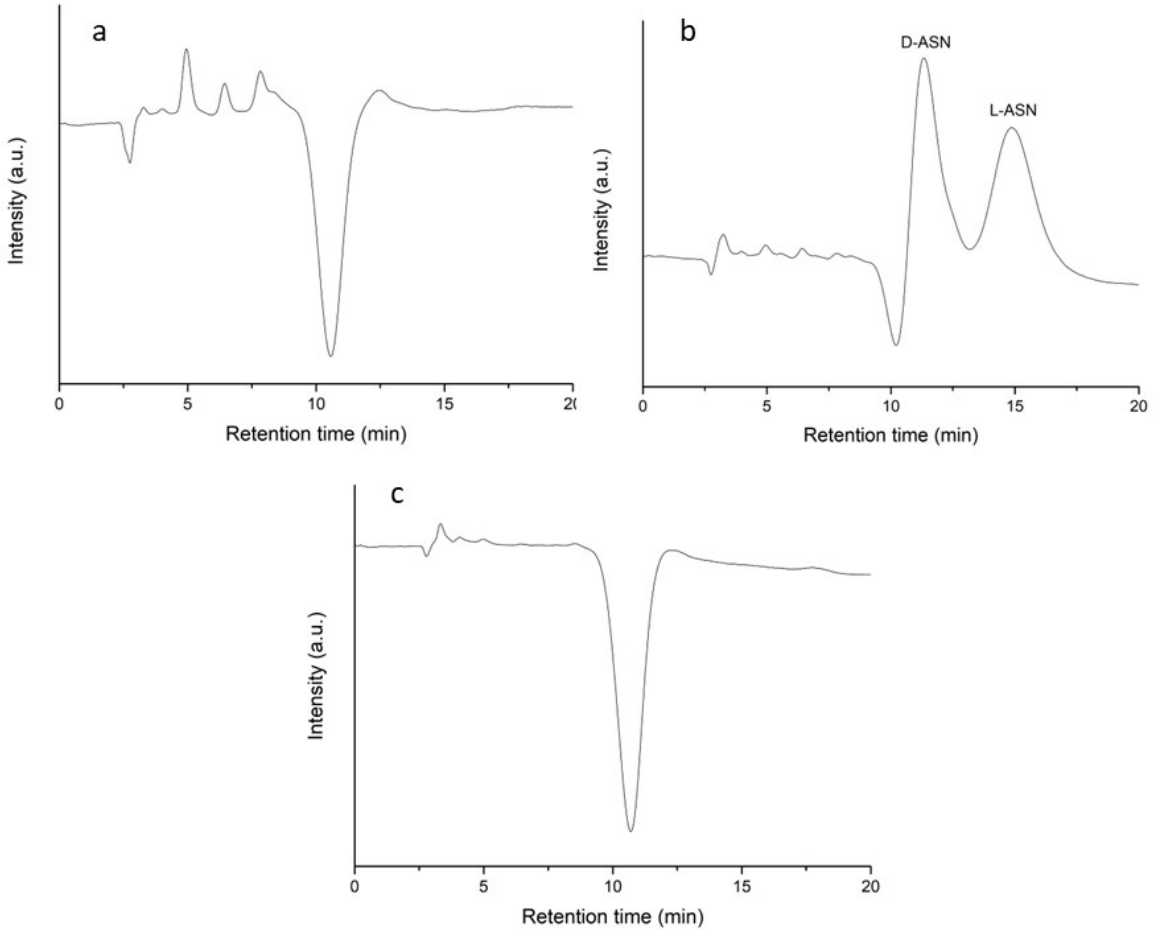


Figure S5 HPLC chromatograms (detection at 254 nm) of the solution used for washing the polymer film for a) 1h b) 4h and c) 12 h. Note that the large negative peak at 11 min is the solvent peak.

6.2) Fourier transform infrared (FITR)

The BT_2T_4 oligomer film exhibits signals at ~3060 cm^{-1} (anti-symmetrical stretching of C_β-H), ~1455 cm^{-1} and ~1420 cm^{-1} (symmetrical stretching of C=C in the aromatic rings), 1316 cm^{-1} (C_β-C_β stretching), 1157 cm^{-1}, 794–790 cm^{-1} (out-of-plane bending of C_β-H) and 760 cm^{-1} (anti-asymmetrical ring deformation of C-S) as presented in the figure below. Note that these signals match the previously reported characterization performed by *Arnaboldi et.al*,[4]

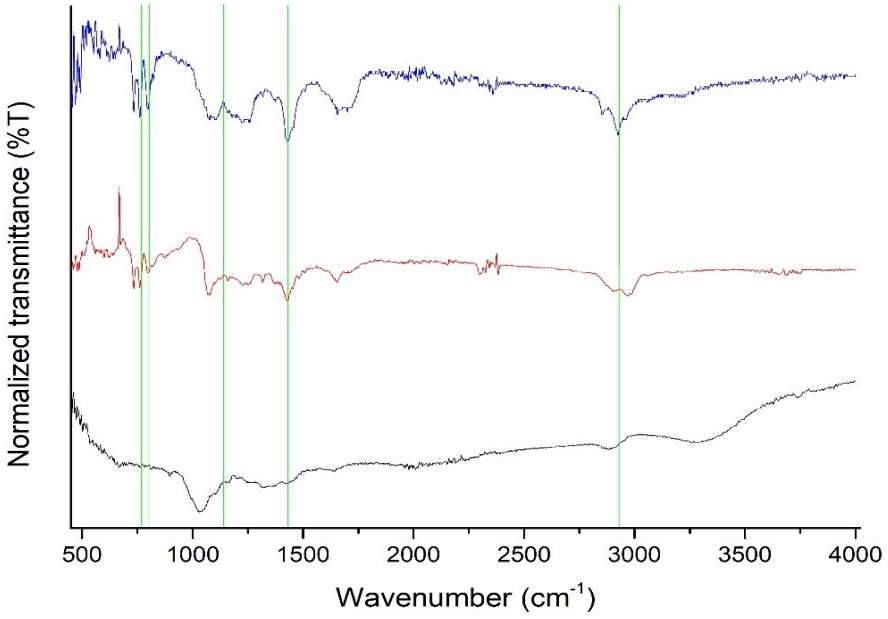


Figure S6 FTIR of the pristine (red) and reused (blue) oligomer films. The spectrum of the unmodified substrate (bare gold) is shown in black.

7) Chiral resolution of *DL*-ASN·H_2O using reused/undoped oligo-(*R*)-/(*S*)-BT_2T_4.

<u>Crystallization yield calculation</u>

The relative yield (with respect to the initial supersaturation) and the absolute yield are calculated using e.q. 1 and 2, respectively. Both yields are reported in Table S4, S5 and S6;

$$\textit{\% Relative yield} = \frac{mass\ of\ obtained\ prefered\ enantiomer}{mass\ of\ avaliable\ supersaturation\ of\ prefered\ enantiomer} \times 100\% \quad (1)$$

$$\textit{\% Absolute yield} = \frac{mass\ of\ obtained\ prefered\ enantiomer}{initial\ mass\ \ of\ prefered\ enantiomer} \times 100\% \quad (2)$$

Where the mass of obtained preferred enantiomer is calculated from HPLC

Mass of available supersaturation = initial conc. (*L*-ASN) – saturated conc. (*L*-ASN, 17 °C)

$$= 0.038 g/g_{sol} - 0.02 g/g_{sol}^{6} = 0.018 g/g_{sol}$$

Since the amount of *DL*-ASN solution used in each crystallization batch is 2.5 ml, thus the mass of available supersaturation is (0.018 g *L*-ASN/g_{sol})* 2.5 g_{sol} = 0.045 g *L*-ASN

Table S2 Enantiomeric excess of the crystals formed outside of the oligomer template.

Experimental	Crystal observations	% *ee*
S-BT_2T_4	crystals on the back of the template	-7.40
S-BT_2T_4	crystals on the back of the template	-6.59
S-BT_2T_4	crystals on the back of the template	-0.26
S-BT_2T_4	SC in the bulk1	+100.00
	SC in the bulk2	-97.42
	SC in the bulk3	+100.00
R-BT_2T_4	one SC in the bulk	+100.00
R-BT_2T_4	one SC in the bulk	+100.00
R-BT_2T_4	very big SC in Bulk	-96.02

*The (+) symbol represents an excess in *D*-ASN and (-) symbol represents an excess in *L*-ASN.

Table S3 Enantiomeric excess from a chiral resolution experiment with *DL*-ASN·H_2O using a non-chiral template, oligo-*(RS)*-BT_2T_4, after 21 h of crystallization.

Experimental	Crystal observations	% *ee*
1	Several crystals on template	+65.92
2	Five crystals on template	+6.82
3	Seven crystals on template	-3.87
4	Several crystals on template	-48.53
5	Several crystals on template	+2.93

*The (+) symbol represents an excess in *D*-ASN and (-) symbol represents an excess in *L*-ASN.

Table S4 Enantiomeric excess and yield from a chiral resolution experiment with *DL*-ASN·H_2O using reused/undoped oligo-*(R)-/(S)*-BT_2T_4, after 21 h of crystallization.

Reused cycles	Oligomer film	Crystal observations	% *ee*	% Absolute yield of *D*-ASN	% Relative yield of *D*-ASN
Fresh	R1	one single crystal	+100.00	0.18	0.38
	R2	two single crystals	+87.61	0.28	0.59
	R3	one single crystal	+100.00	1.38	2.92
1st	R1	one single crystal	+100.00	0.05	0.10
	R2	one single crystal	+100.00	0.12	0.25
	R3	one single crystal	+100.00	17.52	37.00
2nd	R1	two single crystals	+29.27	0.06	0.12
	R2	several crystals	+100.00	1.44	3.04
	R3	one single crystal	+100.00	1.54	3.25
3rd	R1	one single crystal	+100.00	6.96	14.69
	R2	several crystals	+76.10	10.40	21.96
	R3	one single crystal	+100.00	0.36	0.76

*The (+) symbol represents an excess in *D*-ASN.

Table S5 Enantiomeric excess and yield from a chiral resolution experiment with *DL*-ASN·H_2O using fresh/undoped oligo-*(R)-/(S)*-BT_2T_4, after 21 h of crystallization.

Oligomer film	Crystal observations	% *ee*	% Absolute yield		% Relative yield	
			D	*L*	*D*	*L*
R-BT_2T_4	single crystal cluster	+91.47	34.18	-	72.15	-
R-BT_2T_4	single crystal 1	+100.00	1.44			
	single crystal 2	+100.00	0.88	-	4.90	-
R-BT_2T_4	several crystals	+70.11	0.14	-	0.30	-
R-BT_2T_4	one single crystal	+100.00	1.43	-	3.02	-
S-BT_2T_4	five single crystals	-68.60				
	several crystals	-39.01		0.81	-	1.72
S-BT_2T_4	two single crystals	-93.03		0.90	-	1.90
S-BT_2T_4	one single crystal	-100.00		0.14	-	0.29

S-BT_2T_4	one single crystal	-100.00		0.40	-	0.85

*The (+) symbol represents an excess in *D*-ASN and (-) symbol represents an excess in *L*-ASN.

Table S6 Enantiomeric excess and yield from a chiral resolution experiment with *DL*-ASN·H_2O using fresh/doped oligo-*(R)-/(S)*-BT_2T_4, after 12 h of crystallization.

Oligomer film	Crystal observations	% *ee*	% Absolute yield		% Relative yield	
			D	*L*	*D*	*L*
R-BT_2T_4	several single crystals	+79.64	0.06	-	0.13	-
R-BT_2T_4	several single crystals	+33.06	0.36	-	0.76	-
R-BT_2T_4	one single crystal	+100.00	0.99	-	2.09	-
R-BT_2T_4	two single crystals	+100.00	0.05	-	0.10	-
R-BT_2T_4	one single crystal	+100.00	0.03	-	0.06	-
S-BT_2T_4	one single crystal	-97.00	-	8.27	-	17.47
S-BT_2T_4	one single crystal	-100.00	-	0.43	-	0.91
S-BT_2T_4	one single crystal	-100.00	-	0.08	-	0.16
S-BT_2T_4	one single crystal	-100.00	-	0.36	-	0.76
S-BT_2T_4	one single crystal	-100.00	-	0.89	-	1.87

*The (+) symbol represents an excess in *D*-ASN and (-) symbol represents an excess in *L*-ASN.

8) Chiral resolution of *DL*-Glutamic acid using fresh/undoped using fresh oligo-(*R*)-/(*S*)-BT_2T_4.

Table S7 Enantiomeric excess of the chiral resolution experiments performed with 0.045 g/ml *DL*-Glutamic acid (*DL*-Glu) using fresh/undoped oligo-(*R*)-/(*S*)-BT_2T_4.

The crystallization experiments started at 16 °C and the temperature was continuously decreased to 14 °C with a 1 °C/h cooling rate. Then, the temperature was held at 14 °C for 6 hours.

Oligomer film	Crystal observation	% *ee*
(*S*)-BT_2T_4	seven crystals	-41.62
(*S*)-BT_2T_4	one big + three small crystals	-36.15
(*S*)-BT_2T_4	group of crystals	+2.20
(*S*)-BT_2T_4	one crystal	-4.04
(*R*)-BT_2T_4	several crystals	+34.18
(*R*)-BT_2T_4	one big + two small of crystals	+87.28
(*R*)-BT_2T_4	one crystal	+98.25
(*R*)-BT_2T_4	one big + several small crystals	+71.24
bare gold	several crystals	+1.11

*The (-) symbol represents an excess in *D*-Glu and the (+) symbol represents an excess in *L*-Glu.

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