Supplementary Information

The dynamic chromatographic behavior of tri-O-thymotide on HPLC chiral stationary phases.

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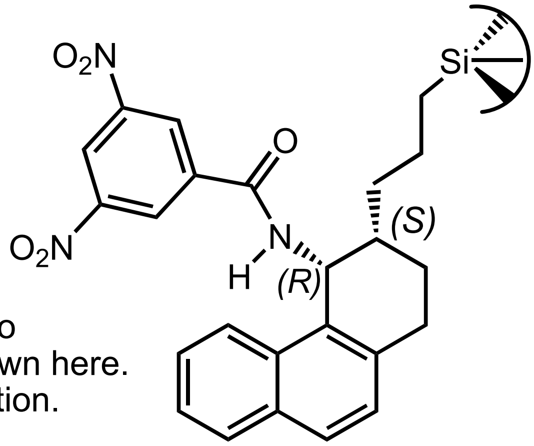
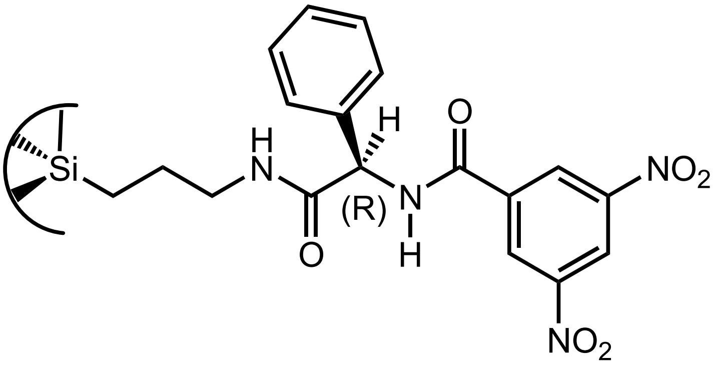
Figure S1. Structures of the chiral stationary phases **CSP1-3**

CSP1

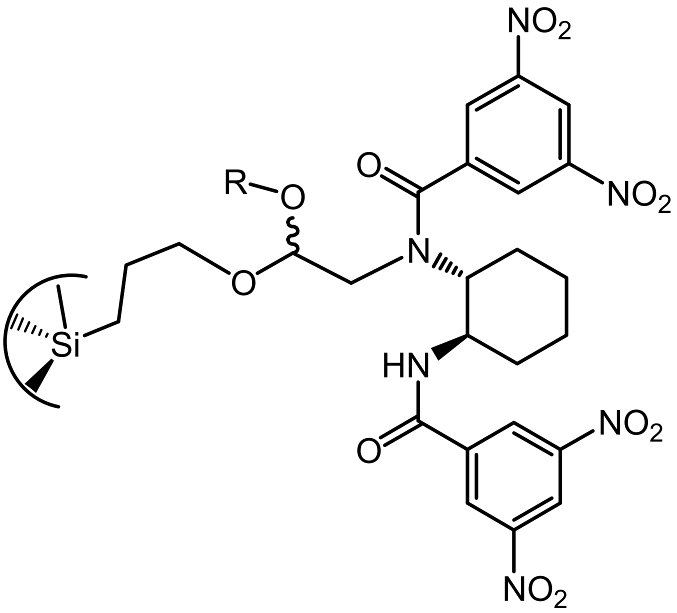


The old R,R configurational notation used in the text has been corrected to the 3R,4S configuration notation shown here. Commercial CSPs have the old notation.

CSP2

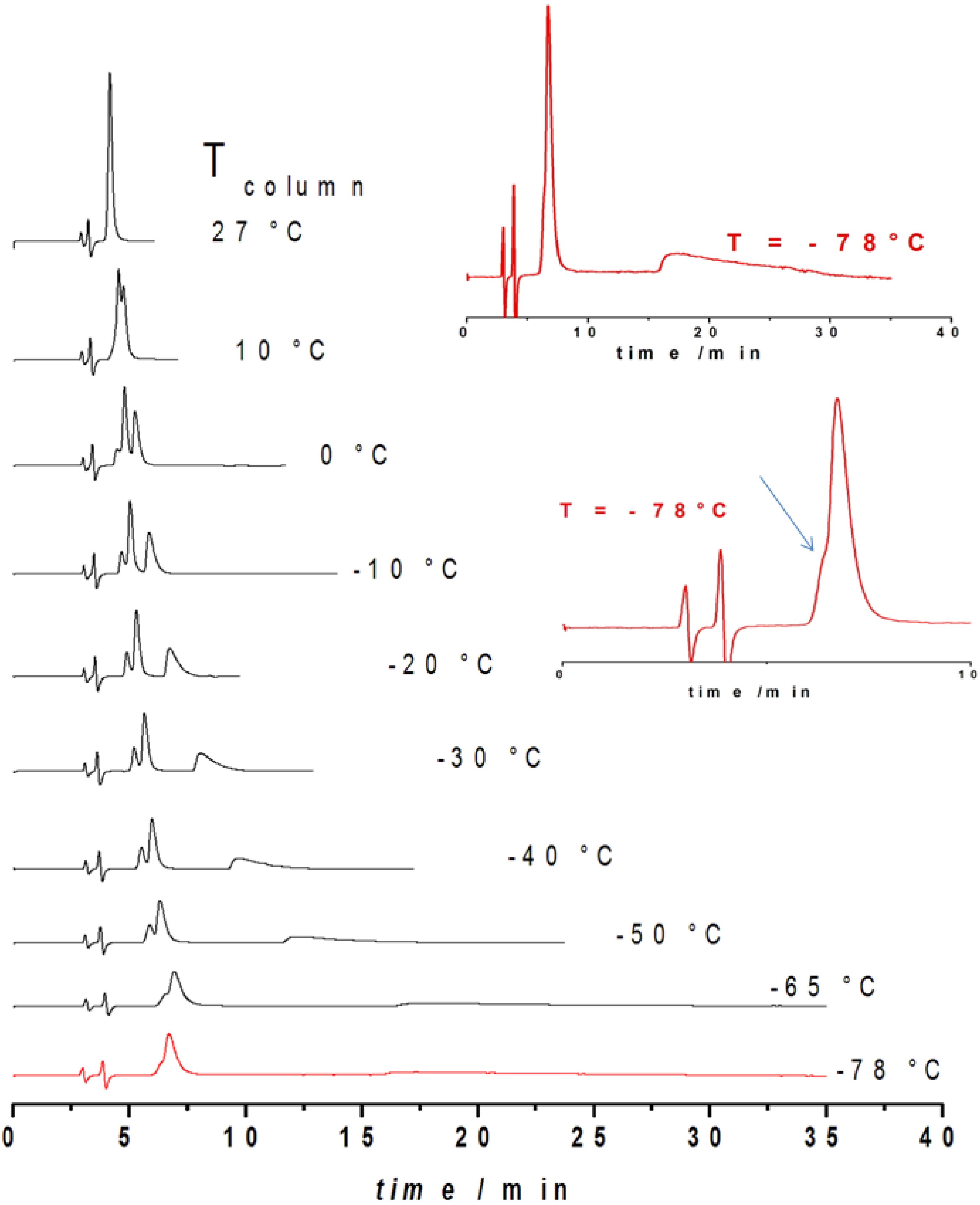


CSP3



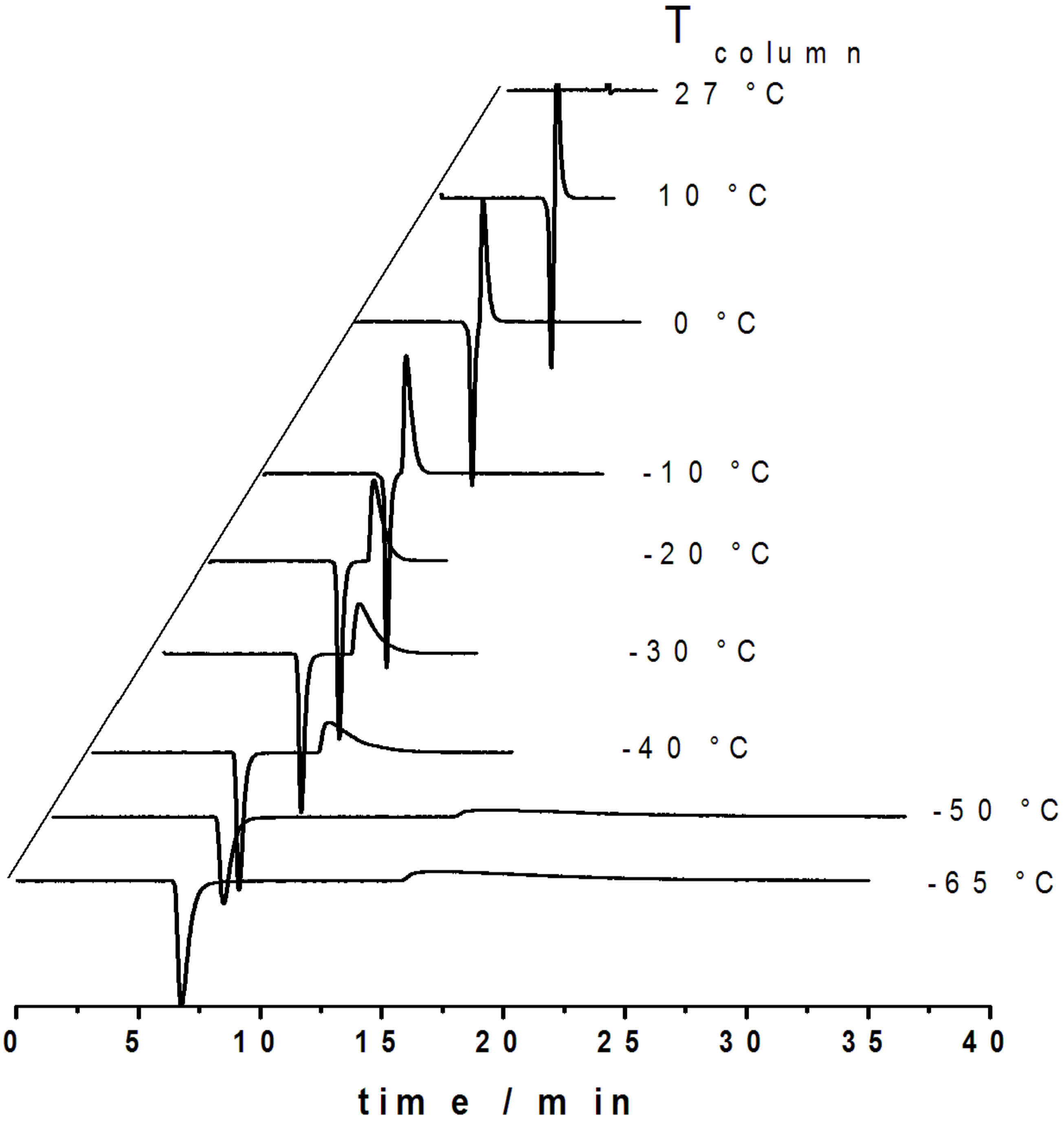
(R,R) configuration shown. The racemic version has been used in this work

Figure S2. Variable temperature HPLC of TOT on **CSP1** with UV detection.



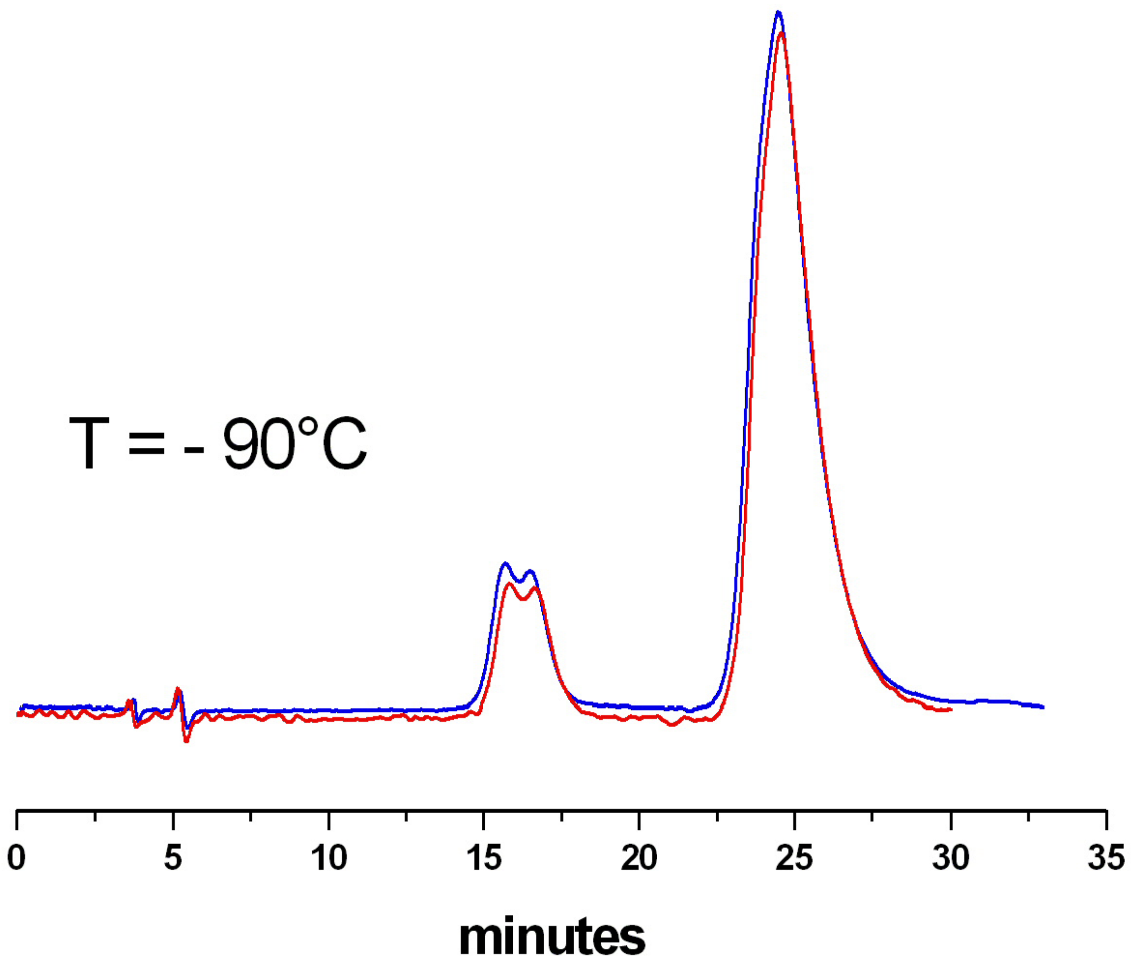
Column: 250*4.6 mm ID; eluent: hexane/CH_2Cl_2/2-propanol 40/50/10; flow rate 1.0 mL min^{-1}; UV detection at 245 nm. Insets show the peaks overlapping of C1 and 1st eluted C3 TOT.

Figure S3. Variable temperature HPLC of TOT on **CSP1** with CD detection.



Column: 250*4.6 mm ID; eluent: hexane/CH_2Cl_2/2-propanol 40/50/10; flow rate 1.0 mL min^{-1}; CD detection at 245 nm

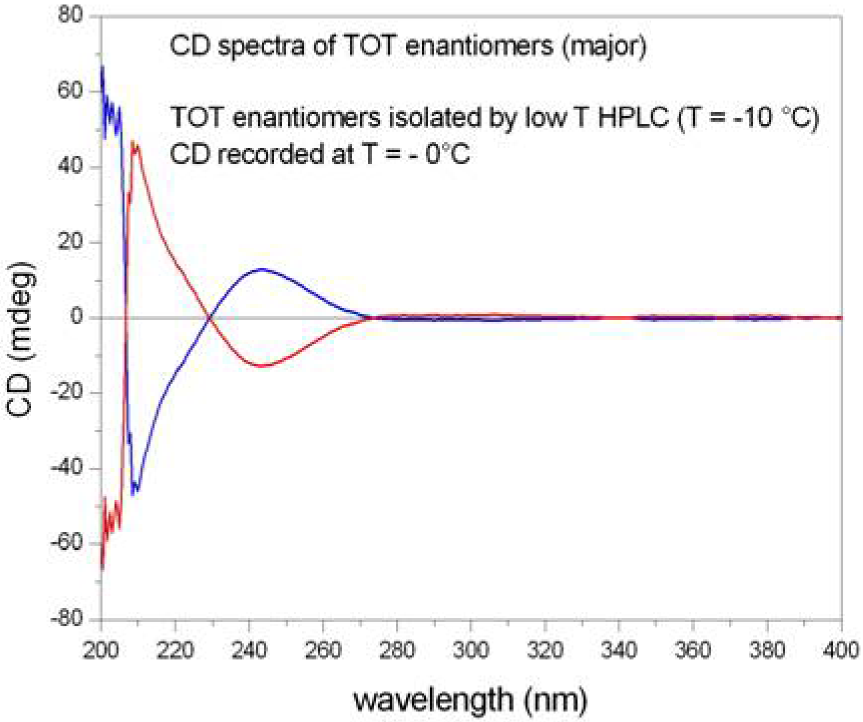
Figure S4. Cryo HPLC of TOT on **CSP2**.

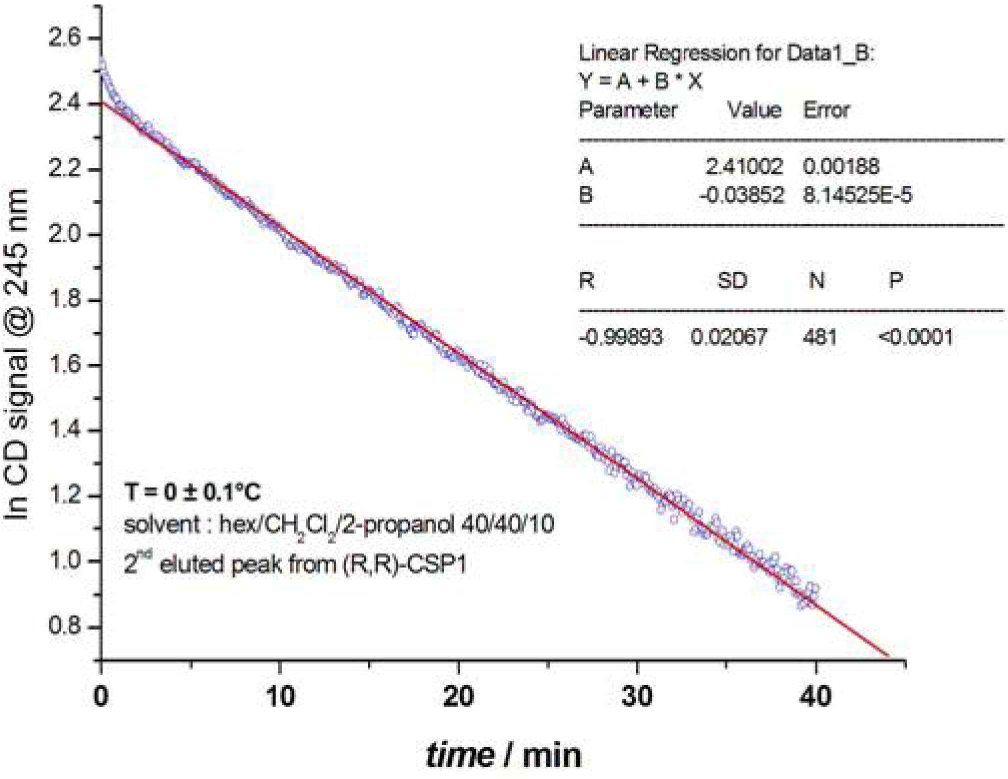


Two replicate overlaid HPLC runs at -90°C. **CSP2** has no selectivity for the major, propeller **pC_3**-TOT species and a low selectivity for the minor, helical **hC_1**-TOT species that are marginally resolved.

Column: 250*4.6 mm ID; eluent: hexane/CH_2Cl_2/2-propanol 40/50/10; flow rate 1.0 mL min^{-1}; UV detection at 245 nm.

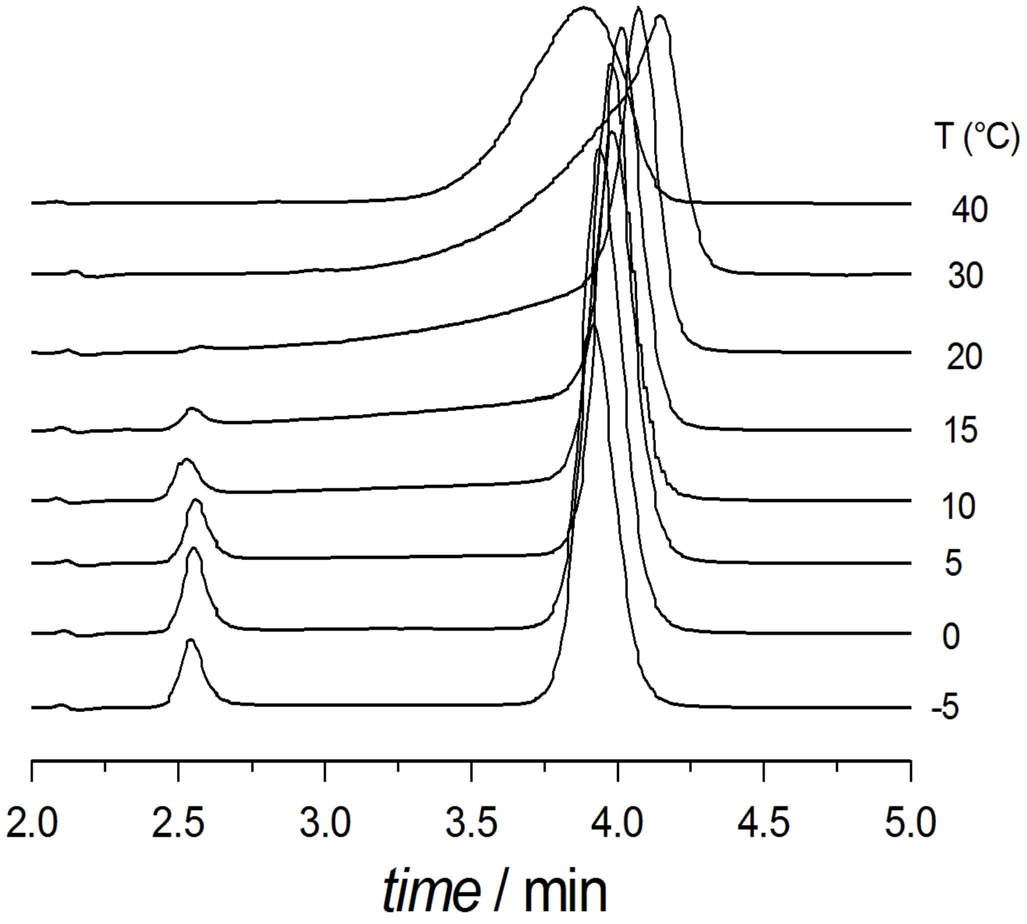
Figure S5. CD spectra of the propeller **pC_3-TOT** enantiomers at T = 0 °C. Red line: 1st eluted enantiomer; blue line: 2nd eluted enantiomer.





Decay of CD signal for the 2nd eluted enantiomer as a function of time at T = 0 °C.

Figure S6. Variable temperature HPLC of TOT on **racemic CSP3.**



Column: 250*4.0 mm ID; eluent: hexane/CH_2Cl_2/2-propanol 40/40/10; flow rate 1.0 mL min^{-1}; UV detection at 245 nm.

Experimental section

Low-Temperature Dynamic HPLC.

Columns packed with **CSPs 1-3** are available from Regis Technologies, Morton Groove, IL.

Cryogenic HPLC was performed placing the column in a dry-ice/acetone or dry-ice/diethyl ether cooling bath, with a 1 m long inlet capillary wrapped around the column to ensure thermal equilibration of the mobile phase. Temperatures were maintained within ± 0.5 °C for at least two consecutive, replicate analysis.

Molecular Modeling Calculations.

Conformational search of **TOT** was carried out by the Batchmin computer program, version 4.5 (Columbia University, NY), using the following options: MM2* Force Field, Montecarlo stochastic algorithm with 5000 generated structures, 100 most stable structures retained among all those obtained from the search, minimization by PR conjugate gradient. All the rotatable bonds resulting after temporary opening of the molecular cycle (eight torsional angles) were explored. The obtained geometries were analyzed by the home made computer program C.A.T. [s1] to exclude twin molecules and to make clusters based on energetic and geometric criteria. From this preliminary procedure fifteen geometries of **TOT** were obtained within an energetic window of 5 kcal mol^{-1} from the global minimum. Seven among these conformers display very similar geometries of C_3 symmetry, with all the carbonyls of the three ester groups placed at the same side of the macrocycle. The other eight conformers, also very similar one to each other, are asymmetric and display only two of the three carbonyl groups at the same side of the macrocycle. The most stable conformers of each of these two ensembles have then been further optimized by DFT calculations based on the BLYP method with the QZ4P large core basis set, as implemented in the Amsterdam Density Functional (ADF) package v. 2007.01. Solvation energies in dichloromethane were also computed with the same program by the COnductor like Screening MOdel (COSMO), with the cavity defined according to the Solvent Excluding Surface (SES) algorithm. Finally, the CD spectrum of the M enantiomer of the C_3 symmetry structure was calculated with the same method. The options were set at: single point calculation; 40 singlet and triplet excitations; diagonalization method: Davidson.

[S1] (a) Alcaro, S.; Gasparrini, F.; Incani, O.; Mecucci, S.; Misiti, D.; Pierini, M. and Villani, C. *J. Comput. Chem.* 2000, 21, 515. (b) Alcaro, S.; Gasparrini, F.; Incani, O.; Caglioti, L.; Pierini, M.; Villani, C. *J. Comput. Chem.* 2007, 28, 1119.