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## ARTICLE

# Chalcogen Bonding Mediated Chiral Recognition and Enantioenrichment of Organoselenocyanates

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Chalcogen bonding (ChB) is recognized as an essential driving force in the Group VIA element-containing materials. The multiple  $\sigma$ -holes with high directionality endow ChB with potentials in chiral functions including recognition, sensing and chiroptical materials. Herein we employ ChB to enable chiral sensing and enantioenrichment and separation of organoselenocyanates. A library of organic selenocyanate derivatives were constructed, of which complexation with a chiral phenanthroline conjugated foldamer was driven by ChB aided by hydrogen bonding and  $\pi$ -stacking forces. The deep chiral environment of the foldamer enables binding affinity bias towards enantiopure selenocyanates that give differential spectroscopic output and binding constants. ChB mediated chiral recognition also shows impacts on self-assembly, that the emergence of macroscopic chirality is controlled by the enantiomeric matching effect. Finally, we realized efficient enantioenrichment of the organoselenocyanates utilizing the ChB assisted chiral recognition for the first time, which establishes a new route in the chiral separation of Se containing pharmaceuticals by  $\sigma$ -hole interactions.

## Introduction

Chiral recognition is fundamentally important in chemistry, pharmaceuticals, and materials science, with significant implications in the synthesis and purification of chiral drugs, asymmetric catalysis, and supramolecular chemistry<sup>[1-10]</sup>. The selectivity is benefited by the directionality and synergistic effects from multiple weak forces, while their dynamic nature results in reversibility, enabling excellent adaptivity and tunability. These advantages have promoted the widespread applications of chiral recognition based on non-covalent interactions, such as van der Waals forces, electrostatic interactions, hydrogen bonding (HB), and  $\pi$ - $\pi$  stacking, across various fields, including drug molecule recognition, the design of chiral sensors, chiral separation media, molecular machines, and catalysis<sup>[11-15]</sup>. ChB commonly occurs in the form of EWG-Ch $\cdots$ LB, where EWG refers to an electron-withdrawing group, LB is a Lewis base, and Ch represents a chalcogen element (O, S, Se and Te)<sup>[16, 17]</sup>. The non-covalent interaction between the electropositive  $\sigma$ -hole and the electronegative region of the Lewis base constitutes the ChB. Although ChB has been known for several decades, its application remains largely underexplored and insufficiently utilized. Current research on ChB is mainly focused on areas such as anion transport<sup>[18-22]</sup>, catalysis<sup>[23-27]</sup> and crystal engineering<sup>[28-33]</sup>. In contrast, studies involving its use in chiral recognition and separation are still very limited<sup>[34, 35]</sup>.

Organoselenocyanate compounds have demonstrated promising anticancer activity and have been extensively studied

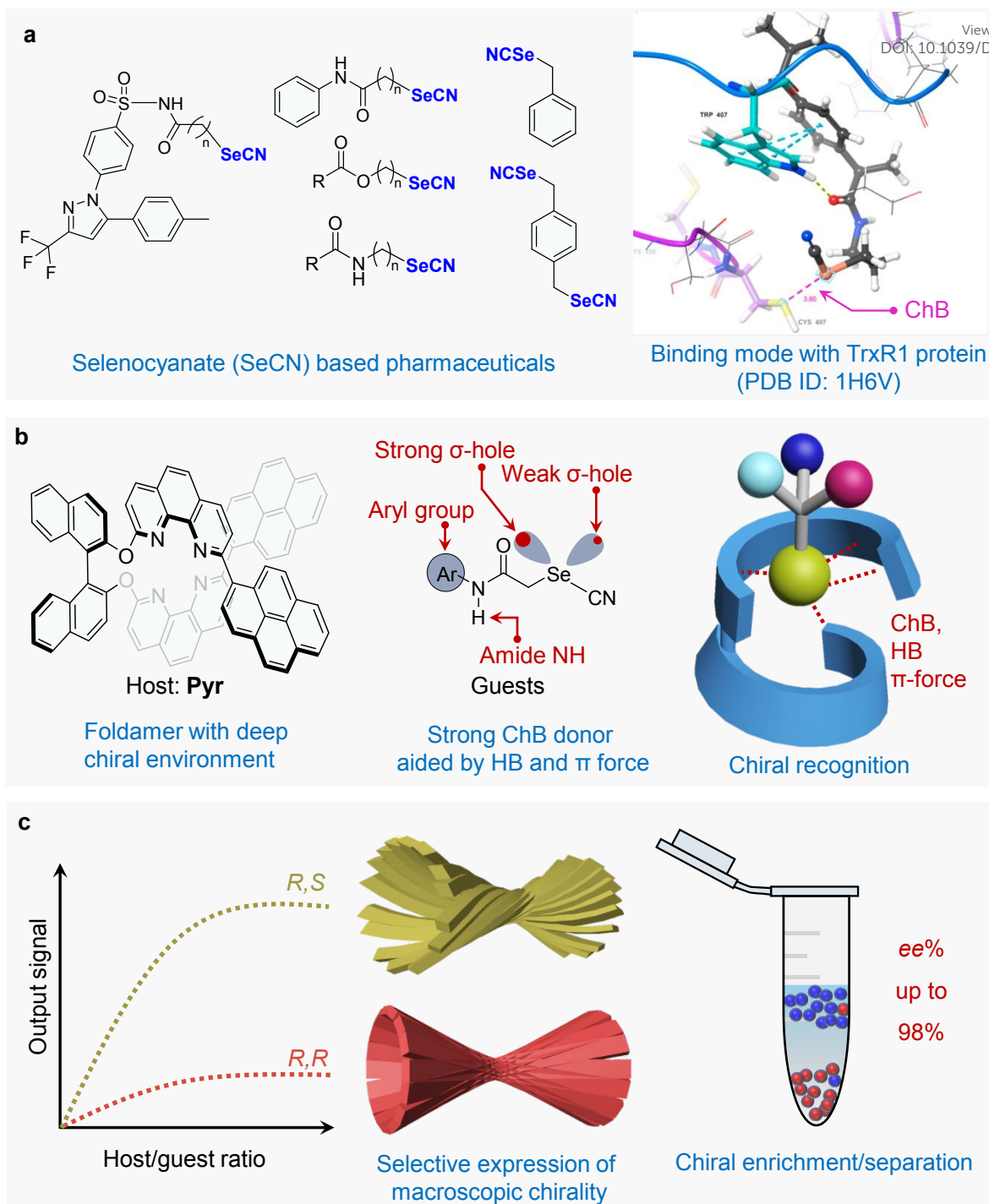
as potential anticancer agents<sup>[36-39]</sup> (Scheme 1a). Meanwhile, organoselenocyanates, as an important class of selenium-containing compounds, exhibit potentials as ChB donors. The strong electron-withdrawing ability of -CN induces a deep  $\sigma$ -hole on Se. The key interactions through which these compounds recognize and bind to biological receptors to exert their pharmacological effects mainly include ChB, HB, and  $\pi$ - $\pi$  interactions (Scheme 1a)<sup>[39]</sup>. Exploring the role of ChB in the supramolecular complexation shall deepen the understanding of drug metabolism and facilitates the enantiomeric sensing and enrichment of organoselenocyanates, which however are still unprecedented so far.

We here aim to mimic the recognition of organoselenocyanate-based anticancer agents by a chiral foldamer (Scheme 1b). The host Pyr comprises of conjugated pyrenes and phenanthroline at a chiral binaphthyl skeleton, which constitutes into a deep chiral environment accomplished by intramolecular folding. The efficient recognition of selenocyanates (Chart 1) is enabled by the ChB aided by the HB and  $\pi$ -stacking forces. The synergistic effects endow with efficient chiral sensing with distinct spectroscopic output bias (Scheme 1c). The chiral matching effects also affect the expression of macroscopic chirality whereby the nanotopology of self-assemblies could be controlled. We also realized efficient enantioenrichment and separation of selenocyanates by the coassembly with the foldamer. The successful application of ChB in chiral recognition and resolution expands the scope of ChB utilization and offers a new perspective for chiral recognition and separation.

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**Scheme 1.** a) Several common selenocyanate (SeCN) anticancer drugs and their binding patterns with TrxR1 protein (PDB ID: 1H6V), adapted with permission from ref. 39. b) Folding host molecule Pyr with deep chiral environment, and the design of guest molecules with strong ChB donors; chiral recognition mode between host and guest through ChB, HB and  $\pi$ -forces. c) Chiral recognition between the host and guest that exhibits different morphologies and achieve chiral separation with high ee% values.



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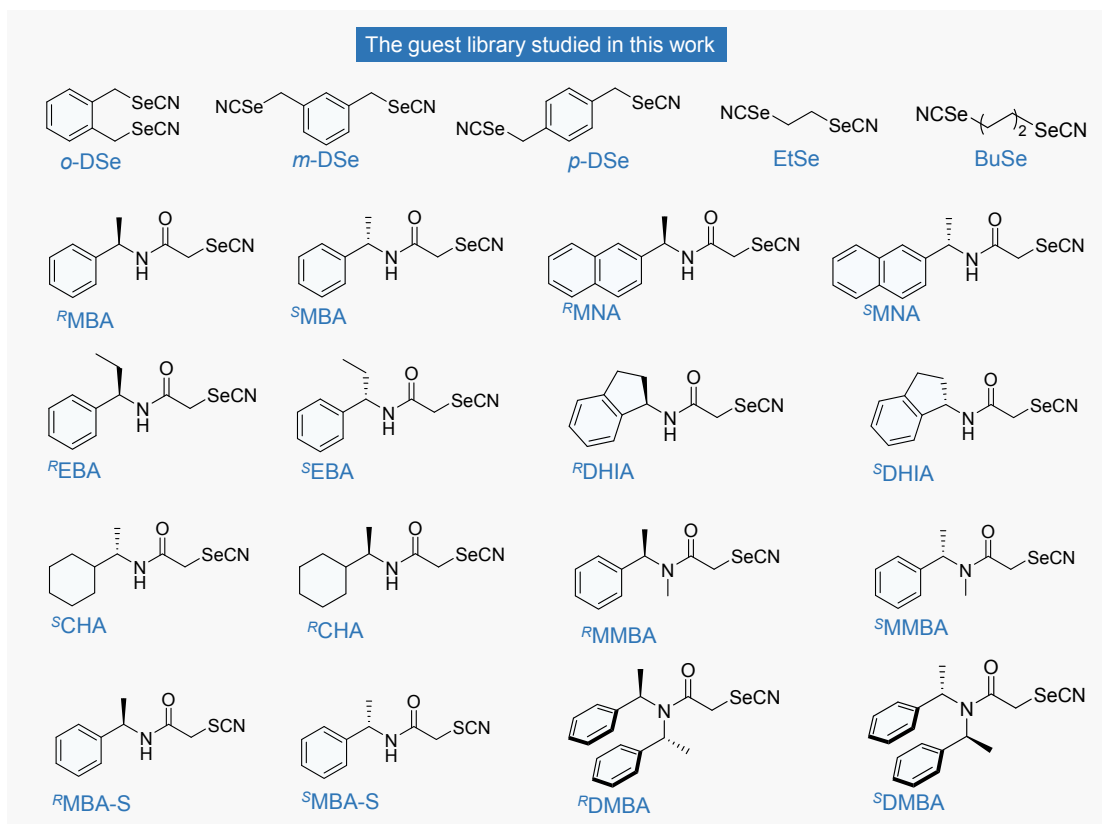


Chart 1. The guest library studied in this work.

## Results and discussion

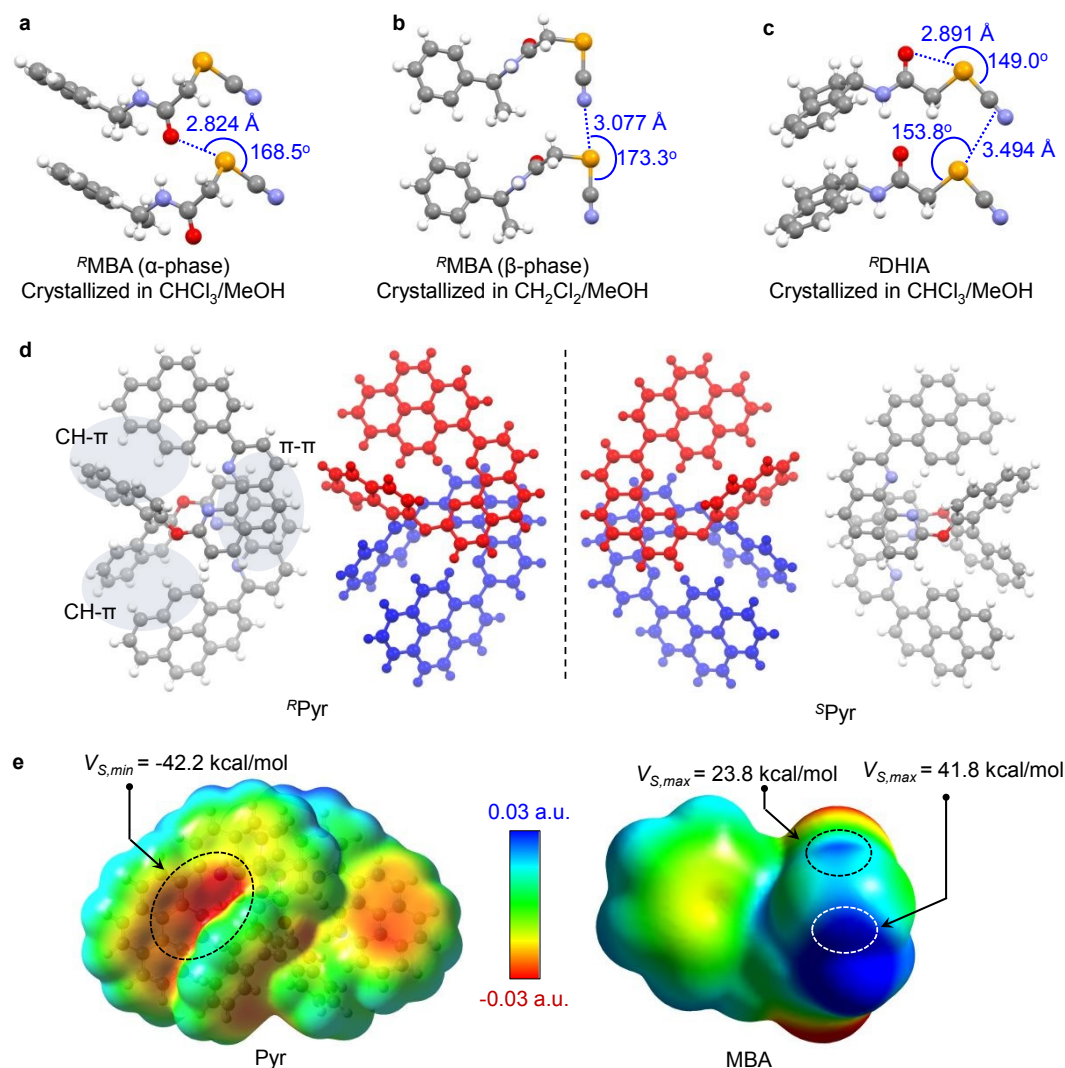
**Synthesis and Structure.** The host Pyr was synthesized through aromatic nucleophilic substitution followed by a Suzuki-Miyaura coupling reaction. For the selenocyanates, the amide intermediates ( $\alpha$ -Cl amides) were obtained via amidation reaction between a chiral amine and chloroacetyl chloride. Then, the guest molecules were synthesized through a nucleophilic substitution reaction with KSeCN. More detailed experimental procedures are provided in the Supplementary Information (SI). The successful synthesis was confirmed through nuclear magnetic resonance ( $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{77}\text{Se}$  NMR) and mass spectrometry (details can be found in the SI). Single crystals of the guest molecules MBA and DHIA, as well as of the host molecule, were successfully obtained via the solvent evaporation method. (Figure 1a-d). Two crystal forms of MBA were obtained by adjusting the solvent conditions. ChB was observed in the crystal structures, including both intramolecular and intermolecular forms. In  $\alpha$ -MBA, intermolecular interactions are formed between the lone pair electrons of the carbonyl group and the strong  $\sigma$ -hole site on Se (2.824 Å,  $\angle\text{C-Se-O} = 168.5^\circ$ ). And the interaction occurs in a linear form between the lone pair electrons on the N of the cyano group and the strong  $\sigma$ -hole on Se in  $\beta$ -MBA (3.077 Å,  $\angle\text{C-Se-N} = 173.3^\circ$ ).

The two forms are respectively crystallized in  $\text{CHCl}_3/\text{MeOH}$  and  $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , and the adaptive feature verifies the great potentials of SeCN in ChB formation. In DHIA, two types of ChB are present including an intramolecular interaction between the carbonyl group and a  $\sigma$ -hole on Se (2.891 Å,  $\angle\text{C-Se-O} = 149.0^\circ$ ), while the other is an intermolecular interaction involving the  $\pi$ -electrons of the cyano group and a weaker  $\sigma$ -hole on Se (3.494 Å,  $\angle\text{C-Se-}\pi = 153.8^\circ$ ). It should be noted that, the  $d_{\text{Se-N}}$  of 3.376 Å is within the sum of van der Waals radius of Se and N (345 pm), yet the lone pair electron of cyano N locates on the perpendicular direction of  $-\text{C}\equiv\text{N}$ . Thus, the Se-N ChB is excluded due to the false angle. The crystal structure of the host Pyr reveals a folded conformation. The two phenanthroline units connected via a binaphthyl linker exhibit  $\pi$ - $\pi$  stacking arrays, while the two pyrene rings attached to the pyrroline units are locked through CH- $\pi$  interactions, exhibiting an overall folding conformation. The molecular electrostatic potential (MESP) was calculated using the Gaussian 16 program at the DFT(B3LYP)/6-311G(d,p) level of theory.<sup>[40]</sup> (Figure 1e,S1) The electron-rich region of Pyr exhibits a negative potential as low as  $-42.2 \text{ kcal}\cdot\text{mol}^{-1}$ , while MBA features a strong  $\sigma$ -hole with  $V_{S,\text{max}} = 41.8 \text{ kcal}\cdot\text{mol}^{-1}$  ( $\sigma^*$  of Se-CN bond) and a weaker  $\sigma$ -hole measuring  $V_{S,\text{max}} = 23.8 \text{ kcal}\cdot\text{mol}^{-1}$  ( $\sigma^*$  of Se-CH<sub>2</sub> bond). Compared to typical benzoselenadiazole



derivatives, which typically exhibit a  $V_{S,max} \approx 20\text{--}30\text{ kcal}\cdot\text{mol}^{-1}$  on Se, these selenocyanate compounds demonstrate significantly enhanced  $\sigma$ -hole potential.<sup>[41, 42]</sup> It exhibits biaxial  $\sigma$ -holes thanks to

the presence of CN and carbonyl group at the  $\alpha$ -position, which enables enhanced ChB bonding with the hosts.



**Figure 1.** a-c) X-ray structures of  $\alpha$ -<sup>o</sup>MBA,  $\beta$ -<sup>o</sup>MBA, and <sup>o</sup>DHIA (CCDC Nos: 2469791, 2469788 and 2469789). d) X-ray structures of Pyr (CCDC No.: 2469790). e) Electrostatic potential maps of the Pyr and MBA.

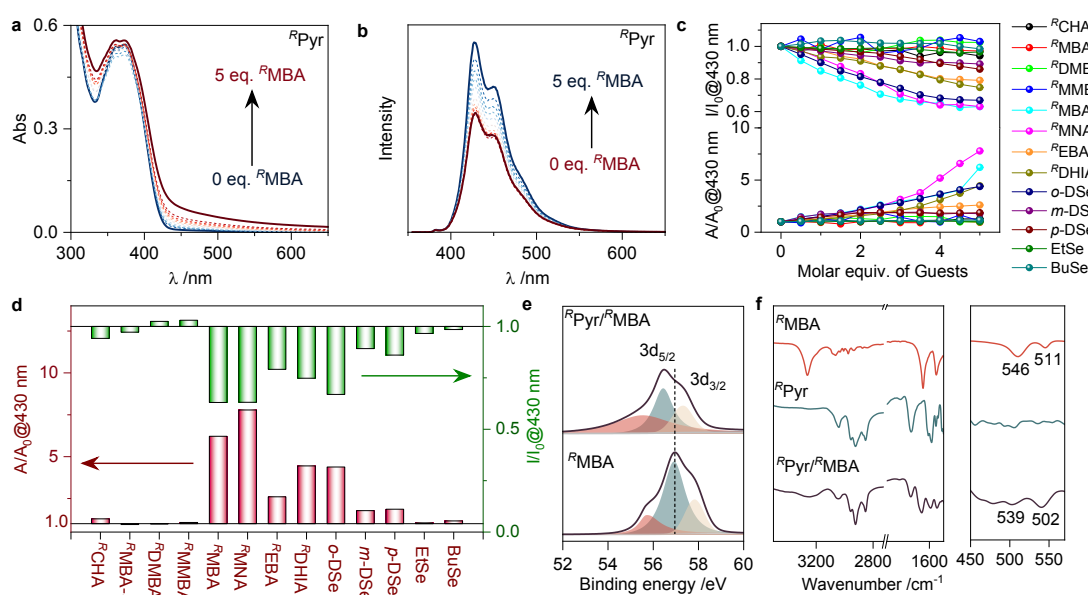
**Interaction between Host and Guest.** The complexation between Pyr and selenocyanate was investigated under solution conditions. Unless otherwise specified, all solution-phase experiments described below were carried out in a chloroform/methylcyclohexane mixture with a volume ratio of 1:39, at a host concentration of 0.05 mM. A titration experiment of the guest was conducted by preparing a series of solutions in which the host concentration was fixed at 0.05 mM while the guest concentration was gradually increased. UV-vis absorption and fluorescence titration experiments were performed in CHCl<sub>3</sub>/methylcyclohexane (MCH) (Figure 2a,b). Upon host-guest complexation, the local electronic environment of the chromophoric units is perturbed by specific noncovalent interactions. This perturbation enhances the effective electronic transition probability, leading to an increase in molar absorptivity. Moreover, upon gradual addition of the guest, host-guest complex formation enhances the

overall aggregation of the system, leading to a corresponding increase in the absorption baseline. Meanwhile, complex formation opens additional nonradiative relaxation pathways in the excited state, resulting in fluorescence quenching. Based on the spectroscopic variations, binding affinities could be clearly determined. In benzyl diselenocyanates and their analogues, including *o*-DSe, *m*-DSe and *p*-DSe (Figure 2c,d), only *o*-DSe exhibited binding activity, while *m*-DSe and *p*-DSe remained inactive. This behavior may be attributed to the shorter distance between the two SeCN groups in *o*-DSe that synergistically enhanced the complexation with phenanthroline groups. Aliphatic selenocyanates BuSe and EtSe with distinct alkyl spacers, were further investigated, which however showed inactive binding with Pyr. These selenocyanates or their analogues indiscriminately show Se-N ChB in solid state X-ray structures<sup>[43-46]</sup>, while in the present diluted (0.05 mM) state



exhibiting significant selectivity based on spectral changes. Subsequently, guest molecules containing aryl and amide units—MBA, EBA, DHIA, and MNA—were investigated. Based on changes in absorption and fluorescence intensities at 430 nm, all four guests exhibited active binding affinity with Pyr (Figure 2c,d). To evaluate the contributions of the three types of interactions, a series of MBA derivatives were designed as control compounds: (1) replacing the phenyl ring with a cyclohexyl group to give CHA, thereby eliminating  $\pi$ - $\pi$  interactions; (2) substituting the -SeCN with the weaker  $\sigma$ -hole donor -SCN to obtain MBA-S, in order to isolate the effect of ChB; and (3) functionalizing the N-H to generate MMBA and DMBA, to assess the necessity of HB. Remarkably, all four control compounds exhibited inactive binding with Pyr, indicating that  $\pi$ - $\pi$  interactions, HB, and ChB are all essential for recognition and binding. ChB serves as the primary directional driving force in the recognition process. The strongly electron-withdrawing -CN substituent induces a

pronounced  $\sigma$ -hole on the Se center, enabling the formation of highly linear and directional ChB interactions with the electron-rich sites of Pyr, thereby anchoring the guest within the chiral cavity. HB further stabilizes the host-guest complex by locking the guest conformation. Specifically, the amide N-H moieties establish hydrogen bonds with the heteroatom sites of Pyr, fixing the guest orientation and promoting stereospecific complementarity within the chiral environment. In addition,  $\pi$ - $\pi$  stacking interactions expand the intermolecular contact interface and enhance chiral discrimination through aromatic matching between the host and guest frameworks, ultimately contributing to increased binding affinity and enantioselectivity. The absence of any one of these interactions leads to a loss of binding activity. These findings confirm that the host is capable of specifically recognizing and binding organoselenocyanates, demonstrating its high selectivity and molecular recognition capability.



**Figure 2.** a) UV-vis titration spectra of <sup>h</sup>MBA with <sup>h</sup>Pyr (0.05 mM, CHCl<sub>3</sub>/methylcyclohexane (MCH), v/v = 1: 39). b) Fluorescence titration spectra of <sup>h</sup>MBA with <sup>h</sup>Pyr (0.05 mM, CHCl<sub>3</sub>/MCH, v/v = 1: 39). c) Absorbance enhancement ratio (A/A<sub>0</sub>) at 430 nm and the fluorescence quenching ratio (I/I<sub>0</sub>) at 430 nm as a function of guest equivalents. d) Histogram of absorbance enhancement ratio (A/A<sub>0</sub>) at 430 nm and the fluorescence quenching ratio (I/I<sub>0</sub>) at 430 nm. e) XPS spectra of <sup>h</sup>MBA before and after interaction with <sup>h</sup>Pyr. The powder used were collected self-assemblies (Pyr/guest 1:1, 0.125 mM, CHCl<sub>3</sub>/MCH, v/v = 1: 39). f) Infrared (IR) spectra of self-assembled <sup>h</sup>MBA, <sup>h</sup>Pyr, and their complex. The powder used were collected self-assemblies (Pyr/guest 1:1, 0.125 mM, CHCl<sub>3</sub>/MCH, v/v = 1: 39).

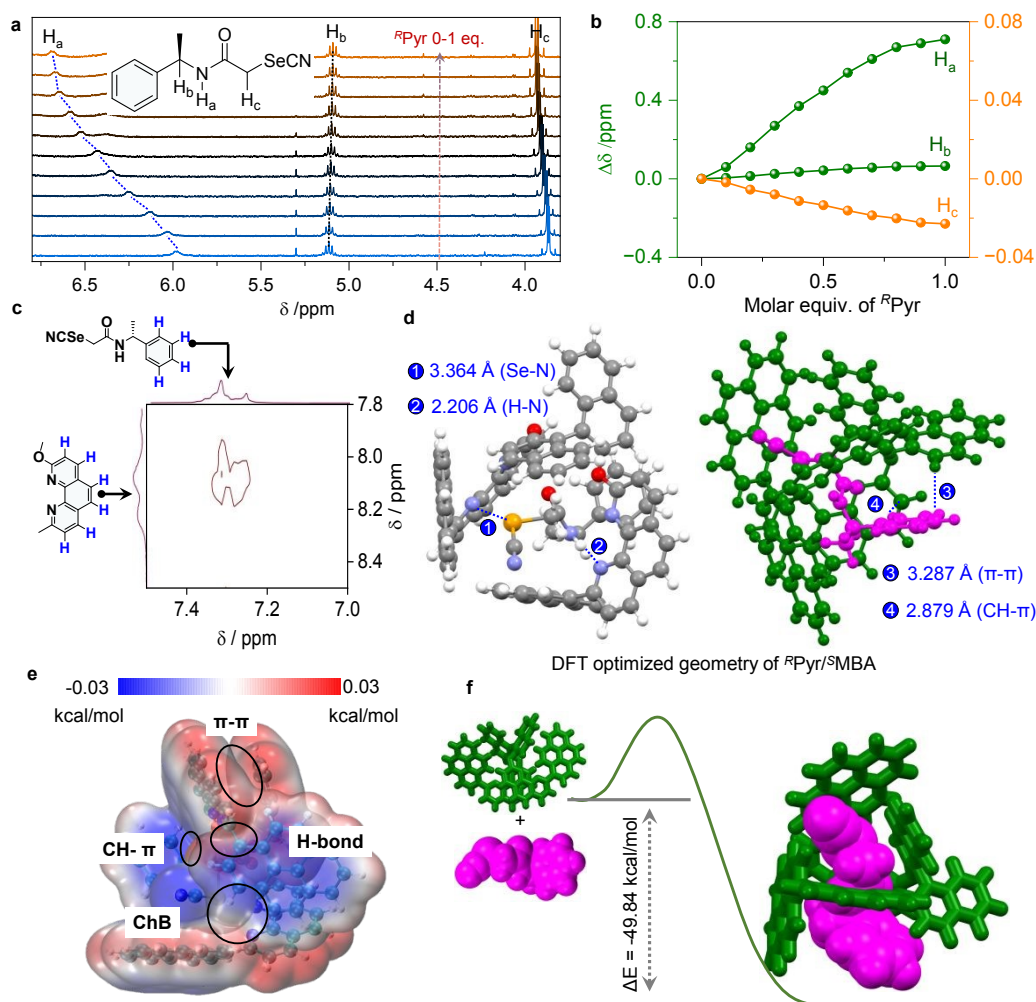
To obtain the assembled state in this solvent system, the solution concentration was increased to 0.125 mM. All subsequent assembly experiments were conducted under the same conditions. Solid powders were obtained by centrifugation under the assembly conditions, followed by X-ray photoelectron spectroscopy (XPS) analysis. (Figure 2e). In the binding energies of the Se 3d<sub>5/2</sub> and 3d<sub>3/2</sub> orbitals, a decrease trending was observed after introducing Pyr, providing further evidence for the formation of ChB. This may be attributed to an increase in the electron cloud density around the Se upon ChB formation. In addition, the  $\sigma$ -hole is partially filled, resulting in a more uniform electron distribution. These changes enhance the shielding effect, thereby reducing the binding strength of the 3d electrons, which is manifested in XPS as a left shift and a decrease in binding energy. Meanwhile, the assembled-state solids

prepared under identical conditions were analyzed by infrared spectroscopy using the KBr pellet method (Figure 2f). The N-H vibration band of amide group appeared at 3262 cm<sup>-1</sup>, and upon interaction with Pyr, this band showed significant broadening, indicating the formation of hydrogen bonding. Correspondingly, the C=O stretching vibration also exhibits noticeable changes after complexation. This can be attributed to the disruption of intramolecular hydrogen bonding and ChB involving the carbonyl oxygen in MBA. Additionally, the Se-C vibrations were observed at 546 and 511 cm<sup>-1</sup>, corresponding to the Se-CN and Se-CH<sub>2</sub> bonds, respectively. After interaction with Pyr, both bands are red-shifted, suggesting a weakening, and lengthening of the Se-C bonds. This observation is consistent with the formation of ChB.



Then, a series of NMR samples were prepared in  $\text{CDCl}_3$ . To facilitate investigation of the NMR spectral changes of the guest, the guest concentration was fixed at 1 mM while the host concentration was gradually increased, and NMR titration experiments were then performed (Figure 3a,b). Pronounced downfield shift was observed in  $\text{H}_a$ , indicating the formation of a hydrogen bond.  $\text{H}_c$  located at the  $\alpha$ -position of the carbonyl group, exhibits a downfield shift due to its proximity to the shielding region of the aromatic ring. This shift is attributed to the ChB interaction between the SeCN group and Pyr, which brings  $\text{H}_c$  closer to the aromatic system. In contrast, the benzylic proton  $\text{H}_b$  showed an upfield shift due to the  $\pi$ - $\pi$  stacking typically requires a parallel arrangement of aromatic rings, while  $\text{H}_b$  lies perpendicular to the aromatic plane, placing it within the shielding regional of the aromatic system. Furthermore, two-dimensional  $^1\text{H}$ - $^1\text{H}$  nuclear Overhauser effect spectroscopy (NOESY) experiments were performed under the same conditions as those used for the NMR titration studies. It confirmed a strong spatial interaction between the aromatic protons of MBA and the protons

on the phenanthroline, providing evidences for the presence of  $\pi$ - $\pi$  interactions with MBA (Figure 3c). The Pyr/MBA complex model was calculated using the Gaussian 16 program at the DFT (B3LYP)/6-311G(d,p) level of theory with the inclusion of D3 dispersion correction, and the binding was evaluated with correction for basis set superposition error (Figure 3d). Phenanthroline-pyrene conjugate constitutes into an overall electron-rich region that Se ( $\sigma$ -hole at the  $\sigma^*$  of Se-CN bond) gives multiple bonds with both N atoms and  $\pi$  planes. Synergistically, N-H and phenyl groups are involved into the HB and  $\pi$ -stacking arrangements, which agrees with the structure-property correlations as discussed above. Subsequently, the MESP between the host and the guest was analyzed under the same basis set (Figure 3e). The existence of ChB, HB and  $\pi$ -stacking interactions can be clearly observed. The binding energy between the host and the guest was also calculated (Figure 3f,S2), and the binding energy between  $^R\text{MBA}$  and  $^R\text{Pyr}$  was approximately -49.8 kcal/mol.



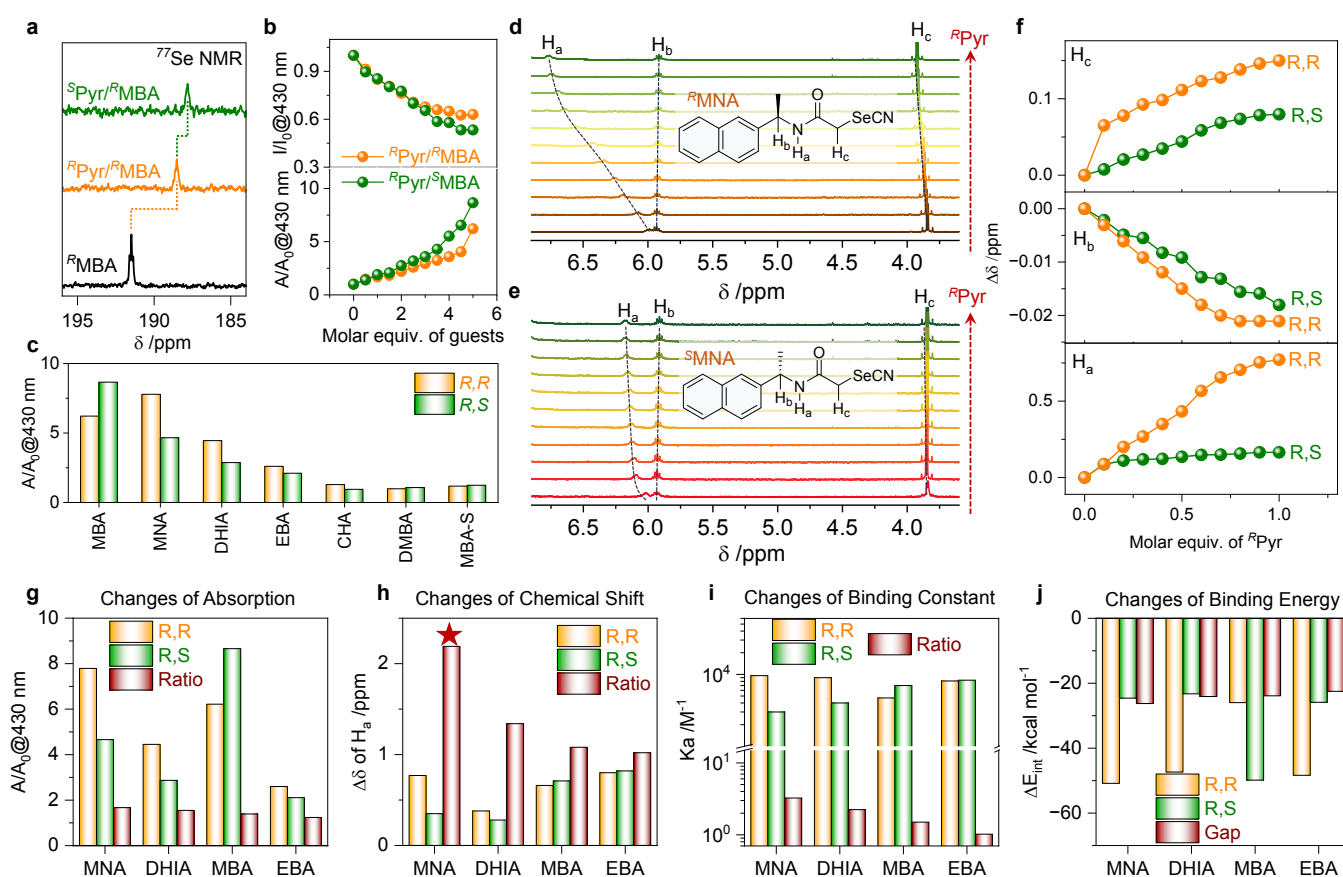
**Figure 3.** a)  $^1\text{H}$  NMR titration spectra of  $^R\text{MBA}$  (1 mM in  $\text{CDCl}_3$ ) with increasing  $^R\text{Pyr}$  from 0 to 1 equiv. b) Plots of chemical shift changes for individual protons of the  $^1\text{H}$  NMR titration as a function of added Pyr equivalents. c) 2D-NOESY spectrum of  $^R\text{MBA}/^R\text{Pyr}$  mixture (1 mM, 1:1, in  $\text{CDCl}_3$ ). d) Optimized binding mode between  $^R\text{MBA}$  and  $^R\text{Pyr}$  calculated at the B3LYP/6-311G (d, p) theory level, partial hydrogens were omitted for clarity. e) Taking  $^R\text{Pyr}$  and  $^R\text{MBA}$  as an example, intermolecular interactions were analyzed using penetration map between molecules. f) Schematic diagram of the binding energy of  $^R\text{Pyr}$  and  $^R\text{MBA}$ .



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**Evaluation of Chirality Recognition.** Samples were prepared following the same procedure, and  $^{77}\text{Se}$  NMR spectroscopy was subsequently performed. In the  $^{77}\text{Se}$  NMR spectra (Figure 4a), upon interaction with  $^5\text{Pyr}$ , the selenium signals of MBA enantiomers shifted upfield towards different positions. It seems that  $^R\text{MBA}$  exhibited a stronger binding affinity toward  $^5\text{Pyr}$  compared to  $^S\text{MBA}$ . These observations provide further evidences for the presence of ChB, also indicating a chiral recognition and matching effect. Then, UV-vis absorption and fluorescence titration experiments were further conducted on different enantiomers (Figure 4b, S16-S23). A clear difference of absorption variations ( $A/A_0$ ) between  $^R\text{Pyr}$  and MBA enantiomers was observed. Consistent with the  $^{77}\text{Se}$  NMR

results,  $^R\text{Pyr}$  exhibited stronger binding affinity toward  $^S\text{MBA}$ ,  $^R\text{MNA}$ ,  $^R\text{EBA}$  and  $^R\text{DHIA}$  respectively, which may result from a mismatch between the spatial specificity required by Pyr and the conventional stereochemical naming rules. Nevertheless, this discrepancy does not affect the validity of the subsequent investigations. The absorption enhancement ratios of all chiral guests after interaction with  $^R\text{Pyr}$  were quantified and summarized in Figure 4c. To evaluate the chiral recognition capability of Pyr for each guest, the ratio of absorption enhancement between the two enantiomers of the same guest was calculated (Figure 4g). The resulting order of chiral recognition was:  $\text{MNA} > \text{DHIA} > \text{MBA} > \text{EBA}$ .



**Figure 4.** Enantiomeric selectivity. a)  $^{77}\text{Se}$  NMR spectra of MBA before and after interaction with Pyr (1 mM in  $\text{CDCl}_3$ ). b)  $A/A_0$  and  $I/I_0$  as a function of guest concentration. c) Absorption enhancement for each guest. d,e)  $^1\text{H}$  NMR titration spectra of  $^R\text{MNA}$  and  $^S\text{MNA}$  (0.01 mM in  $\text{CDCl}_3$ ) with increasing  $^R\text{Pyr}$  from 0 to 1 equiv. f) Plot of chemical shift changes for individual protons during the NMR titration experiment as a function of Pyr equivalents. Chiral recognition ability recorded by several parameters including changes of g) absorption, h) chemical shift, i) binding constant ( $K_a$ ) and j) binding energies calculated at the B3LYP/6-311(d, p) level with the basis set superposition error (BSSE) correction. (Ratio =  $\text{Value}_{R,R} / \text{Value}_{R,S}$ , Gap =  $\Delta E_{R,R} - \Delta E_{R,S}$ )

Further evaluation was carried out using  $^1\text{H}$  NMR titration experiments (Figure 4d,e), which is consistent with the UV-vis and fluorescence titrations. Among all guests, MNA exhibited the most significant chemical shift changes upon interaction with Pyr. The chemical shift variation of MNA was plotted against the equivalents

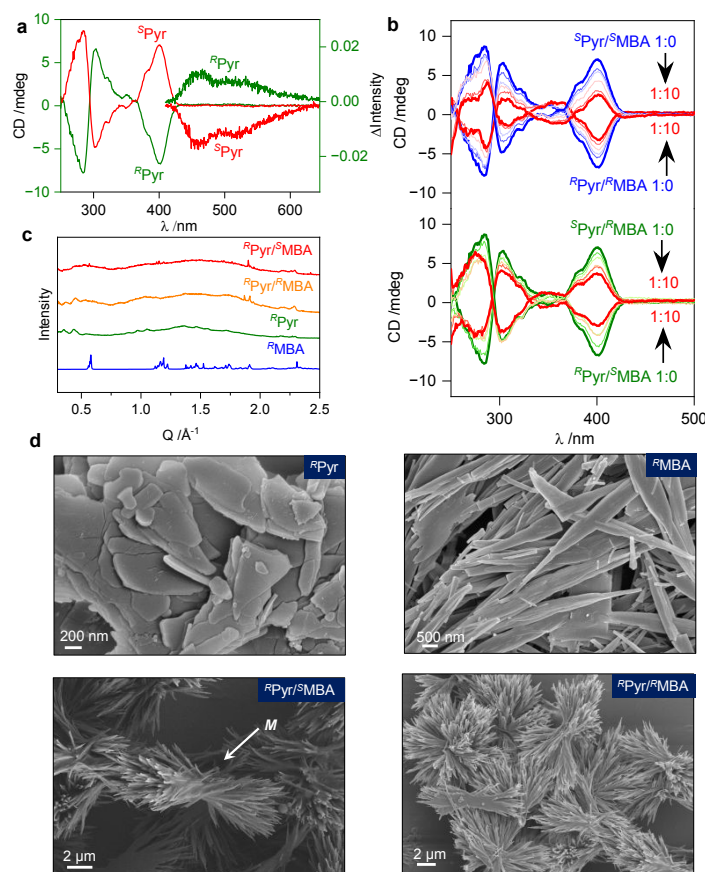
of added host (Figure 4f). Chemical shift changes for each guest were also quantified, and the ratio between the two enantiomers of each guest was used to assess the degree of chiral recognition which gave the same order with UV-vis/fluorescence titration experiments (Figure 4g). Moreover, the chemical shift changes of  $\text{H}_a$  were fitted



to obtain the  $K_a$  between the host and each guest (Figure 4h,i). The ratio of  $K_a$  values for the two enantiomers of each guest was then used to evaluate the chiral recognition ability. All host-guest combinations exhibit comparable binding strengths ( $K \approx 10^3$ ). Among them,  $R$ MNA/ $R$ Pyr displays the strongest binding affinity, with a  $K$  value of  $9.6 \times 10^3$ . Notably, MNA also shows the highest selectivity, with a binding ratio of  $K_{R,R}/K_{R,S} = 3.25$ . This behavior originates from the combined effects of molecular shape and the available aromatic surface of MNA. Local aromatic regions of MNA participate in  $\pi$ - $\pi$  and C-H $\cdots$  $\pi$  interactions with the host. Moreover, this conclusion is further supported by MESP analysis (Figure S8-15) and by the enhanced contribution of dispersion interactions revealed in the energy decomposition analysis (Table S1). For spatially matched guests, the larger aromatic surface allows more extensive dispersive interactions, leading to stronger binding, while the increased steric bulk enhances chiral selectivity. In contrast, spatial mismatch leads to inefficient aromatic overlap, increased steric repulsion, and weakened binding. Next is DHIA, which exhibits a binding ratio of 2.24. We speculate that this may be due to the host's preferential recognition of planar structures, leading to higher selectivity for DHIA

compared to MBA and EBA. MBA and EBA follow, with binding ratios of 1.50 and 1.02, respectively. This trend is consistent with the order observed in both UV-vis absorption and NMR chemical shift experiments.

Subsequently, further validation of this conclusion was achieved through computational simulations using the Gaussian 16 program at the DFT (B3LYP)/6-311G(d,p) level of theory with D3 dispersion correction, combined with basis set superposition error (BSSE). The chiral recognition ability was evaluated by comparing the binding energy gaps between the two enantiomers of each guest (Figure 4j). The same order was obtained further confirming the reliability of this sequence. Among the guests, MNA exhibited the highest chiral selectivity, which can likely be attributed to its larger  $\pi$ -conjugated system. The importance of the aryl structure was already demonstrated in the earlier comparison between DSe and BuSe. The order DHIA > MBA > EBA may be explained by differences in steric hindrance. Smaller steric bulk facilitates deeper penetration of the guest into the chiral electronic environment of the host cavity, thereby enhancing the enantiomeric effect.



**Figure 5.** a) Circular dichroism (CD, 0.05 mM,  $\text{CHCl}_3/\text{MCH}$ ,  $v/v = 1:39$ ) and circularly polarized luminescence (CPL) of Pyr. CPL measurements were conducted using thin films prepared using assembly. And the assembly was obtained by mixing Pyr and the guest at a 1:1 molar ratio with a concentration of 0.125 mM in a  $\text{CHCl}_3/\text{MCH}$  ( $v/v = 1:39$ ) solvent system. b) CD titration spectra of Pyr with different enantiomers of MBA. c) The GIXS patterns and integral curves. Conditions are same as CPL. d) Morphologies of different assemblies captured by scanning electron microscopy (SEM). Conditions same as CPL.

**Study on Chiroptical properties.** Samples were prepared under solution-phase conditions for circular dichroism (CD) measurements. The chiroptical properties were studied. In the CD spectra, exciton-

coupled bands were observed around 400 nm, corresponding to the characteristic absorption peaks of Pyr (Figure 5a). Based on the single-crystal structure of Pyr, the appearance of exciton-type signals



in the CD spectrum can be attributed to the close spatial arrangement of aromatic systems within Pyr, which leads to exciton coupling with a chiral orientation of the transition dipole moments. Upon the addition of MBA to Pyr, different chiral combinations exhibited distinct evolutions (Figure 5b). Additionally, a new signal emerged at 430 nm. In the corresponding absorption spectra, a general decrease trending was also observed; however, a signal inversion occurred at 350 nm, and no new peak appeared at 430 nm. The combinations with different absolute chirality exhibited more pronounced exciton-type signals, which may originate from the stronger binding activity between <sup>R</sup>Pyr and <sup>S</sup>MBA. This enhanced binding affinity brings the aromatic units into closer proximity, thereby resulting in more distinct excitonic features. These distinct patterns of supramolecular evolution suggest the presence of two different binding modes or aggregation behaviors within the supramolecular complexes.

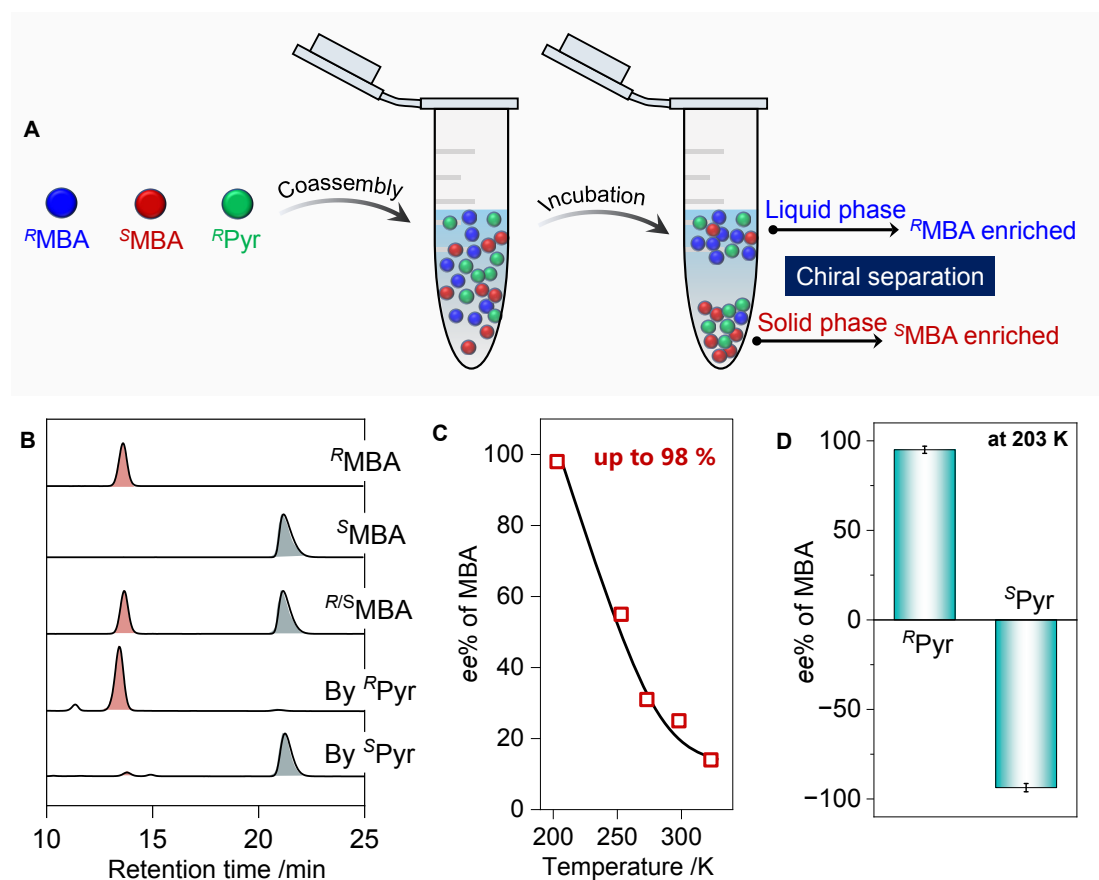
In addition, to investigate the relationship between supramolecular structures and chiral luminescence, samples were prepared under assembly conditions for circularly polarized luminescence (CPL) measurements (Figure 5a). In the CPL spectra, chiral luminescence was observed at wavelengths corresponding to the emission of the assemblies. Specifically, <sup>R</sup>Pyr exhibited positive CPL, while <sup>S</sup>Pyr showed negative CPL, with a  $glum$  value of approximately  $\pm 0.015$ . Furthermore, CPL measurements of the host-guest assemblies revealed a general trend (Figure S45-S48). For MBA, a slight increase in  $glum$  was observed in homochiral combinations, whereas heterochiral combinations led to a complete inversion of the CPL signal.

**Characterization of Morphology and Topology.** To further explore the possible supramolecular arrangements, samples prepared under assembly conditions were deposited onto silicon substrates and subjected to grazing-incidence X-ray scattering (GIXS) analysis (Figure 5c). The GIXS patterns show that both Pyr and MBA exhibit well-defined crystalline features. Comparison with the single-crystal data reveals that, under the given assembly conditions, MBA tends to adopt the  $\beta$ -phase. In the coassembly phase, all pristine peaks were replaced by new peaks, implicating the formation of new phase

without inherent self-sorting. Compared to the homochiral combinations (*R/R* or *S/S*), heterochiral combinations (*R/S* or *S/R*) show stronger binding affinity that improves the structural integrity of the complexes and enables the expression of chirality at macroscopic scale. This observation is consistent with their different peaks, and heterochiral coassemblies possess fewer scattering bands corresponding to the elongated one-dimensional extension with higher aspect ratio, which facilitates the emergence of macroscopic chirality.

Samples were prepared using the same procedure as that employed for GIXS measurements, and the self-assembled nanoarchitectures were investigated by scanning electron microscopy (SEM) (Figure 5d). Pyr exhibited a sheet-like morphology, while MBA formed fibrous structures. Upon co-assembly, the resulting structures transformed into thinner fibers that tended to aggregate into higher-order superstructures. In the <sup>R</sup>Pyr/<sup>R</sup>MBA coassemblies show no expression of macroscopic chirality. In contrast, the <sup>R</sup>Pyr/<sup>S</sup>MBA system forms left-handed (M-type) helical assemblies, while the <sup>S</sup>Pyr/<sup>R</sup>MBA gives rise to right-handed (P-type) helices, demonstrating a consistent and reproducible correlation between the molecular chirality of the components and the helical handedness of the resulting assemblies. This helical sense originates from a hierarchical self-assembly process, in which the intrinsic chirality of the Pyr units is retained in locally ordered subunits and subsequently biases their directional stacking, leading to a preferred sense of twist during growth. The accumulation of this biased stacking across length scales ultimately results in macroscopic helices with a well-defined handedness.<sup>[47]</sup> Similar structural differences were also observed in other chiral guest systems, indicating that distinct binding modes exist in different chiral combinations (Figure S49-S52). In light of the observed CPL trends, it is reasonable to infer that the fine fibrous structures within the assemblies enhance the chiral expression of the chromophores, thereby leading to the increased  $glum$  value observed in the <sup>R</sup>Pyr/<sup>R</sup>MBA system. Moreover, the supramolecular helical structures arising from chiral evolution contribute to a secondary level of chiral expression, which accounts for the CPL signal inversion observed in the <sup>R</sup>Pyr/<sup>S</sup>MBA system.





**Figure 6.** a) Schematic illustration of the chiral resolution process. b) HPLC patterns of MBA monomer and the resolution products. c) Summary of enantiomeric excess values of chiral resolution results under different host. d) ee% of MBA separation at different temperatures.  $ee\% = \frac{([R]-[S])}{([R]+[S])}$

**Application of Enantiomer Separation.** An enantiomer separation strategy was developed based on the enantioselective recognition. Using the same solvent system and concentration conditions as those employed during the assembly process, environmental parameters including temperature and aging time were optimized.  $^R\text{Pyr}$  was co-assembled with racemic MBA, followed by treatment via a nanoprecipitation method (Figure 6a). The relevant information is described in detail in the SI. After incubation and centrifugation, the mixture was separated into supernatant and precipitate fractions. And due to the differential binding affinities of  $^R\text{MBA}$  and  $^S\text{MBA}$  toward  $^R\text{Pyr}$ , their distribution between the two fractions diverges accordingly. The enantiomeric excess (ee%) values of both fractions were subsequently determined by high performance liquid chromatography (HPLC) analysis. Racemic MBA and its two enantiomers were analyzed to identify their retention times. The results showed that  $^R\text{MBA}$  eluted at approximately 14 min, while  $^S\text{MBA}$  eluted at around 21 min (Figure 6b). Next, the assembled materials obtained under different temperature were analyzed, and

the corresponding ee% values were summarized in Figure 6c. Temperature-controlled experiments revealed that lower temperatures favor chiral resolution. Temperature-controlled experiments revealed that lower temperatures favor chiral resolution, indicating that the binding specificity is primarily governed by kinetic factors. Among the tested conditions, the best separation was achieved after aging at 203 K for 24 hours, with an ee% up to 98%. The pronounced temperature sensitivity observed in the separation process is likely attributable to the differences in entropy changes during the recognition process. Owing to the deep chiral cavity environment of the host, the two enantiomeric guests undergo distinct recognition and binding modes, which consequently result in different entropy variations upon complex formation. To further elucidate this behavior, the thermodynamic parameters of the systems were investigated. The calculated entropy changes for the formation of the  $^R\text{Pyr}/^R\text{MBA}$  and  $^R\text{Pyr}/^S\text{MBA}$  complexes were  $-230.074 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$  and  $-258.613 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ , respectively. Based on the Gibbs-Helmholtz relationship,  $\Delta G = \Delta H - T\Delta S$ , a smaller  $\Delta S$  value



implies a more pronounced decrease in  $\Delta G$  upon lowering the temperature. Therefore, during the competitive recognition process between  $^R\text{MBA}$  and  $^S\text{MBA}$ , decreasing the temperature thermodynamically favors the binding of  $^R\text{Pyr}$  with  $^S\text{MBA}$ . Consequently, the proportion of  $^S\text{MBA}$  remaining in the liquid phase decreases, which is experimentally manifested as an increase in the ee value. Based on these findings, we developed a chiral recognition and resolution strategy mediated by ChB, addressing the current gap in the application of ChB in this field. This work expands the functional scope of ChB and paves the way for its advancement as a powerful tool in supramolecular science, chemistry, and even the broader natural sciences.

## Conclusions

In summary, we report a chiral recognition and separation strategy of organoselenocyanates based on ChB. To mimic the recognition behavior of biological receptors toward selenocyanate-based agents, a host molecule featuring a deep chiral cavity was rationally designed. Correspondingly, selenocyanate-based guest model compounds were synthesized based on the structural characteristics of the host. Efficient chiral recognition was successfully achieved. And the recognition process was demonstrated to rely on three key noncovalent interactions: HB,  $\pi$ - $\pi$  stacking, and ChB. Furthermore, we investigated the properties of the assembled structures and found that different chiral combinations exhibited distinct supramolecular evolution pathways across various characterization methods. Finally, leveraging the specificity of supramolecular recognition, we innovatively designed a chiral resolution strategy based on ChB, achieving a maximum ee% of 98%. This work offers an alternative perspective for the development of chiral separation methodologies. It highlights the untapped potential of ChB in chiral recognition and separation, and envisions their future as valuable building blocks in the field of supramolecular chemistry.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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