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Enantiodiscrimination and molecular docking study of chiral amines as 2-hydroxynaphthaldimine derivatives using amylose derived chiral selectors

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Abstract: This study describes the liquid chromatographic enantiomer separation of three typical chiral amines (α-methylbenzylamine, 2-amino-4-methyl-1-pentanol, and 1-methylheptylamine) as 2-hydroxynaphthaldimine derivatives using six amylose trisphenylcarbamates derived chiral stationary phases (CSPs). It was observed that the structural nature of three chiral amines and the structures of amylose chiral selectors can affect their chiral recognition ability. Among the three analytes as 2-hydroxynaphthaldimine derivatives, in general, the greatest enantioselectivities of aromatic amine analyte (α-methylbenzylamine) were achieved on amylose trisphenylcarbamate derived CSPs and were followed by amino alcohol analyte (2-amino-4-methyl-1-pentanol), and aliphatic amine analyte (1-methylheptylamine). Also, the enantiodiscrimination abilities obtained on the two CSPs, Chiralpak ID and Chiralpak IF, were selectively higher than the other four amylose trisphenylcarbamate derived CSPs for the studied analytes. The underlying chiral recognition mechanism between 2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives and amylose tris(3,5-dimethylphenylcarbamate) chiral selector of Chiralpak AD-H and Lux Amylose-1 was elucidated by molecular docking study, and it was observed that the intermolecular hydrogen bonding interactions by hydroxyl moiety on the amino alcohol analyte as 2-hydroxynaphthaldimine derivatives were the main interactive forces driving the chiral separation. The obtained binding energies between 2-amino-4-methyl-1-pentanol analyte as 2-hydroxynaphthaldimine derivative and amylose tris(3,5-dimethylphenylcarbamate) chiral selector were in agreement with the experimentally determined enantioseparation and elution order by chiral HPLC.

Key words: amylose trisphenylcarbamate, chiral amine, enantiomer separation, docking simulation, 2-hydroxy-naphthaldimine derivative

1. Introduction

Stereoselectivity is frequently observed in our biological systems due to the inherent chirality of essential biological macromolecules.[1,2] It is well established that the biological or pharmacological

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responses of enantiomers differ when interact with a chiral biological macromolecule.[2-5] Chiral amines are valuable chiral analogues in pharmaceuticals or other chemical industries as they serve as key role as auxiliaries or scaffolds in stereoselective organic synthesis or as building blocks for the production of many pharmaceuticals and biologically active molecules.[5-9] It is reported that approximately 40 % of commonly used drugs in the United States contain chiral amines as the core moieties.[7,8] Given the significance of chiral amines, it is crucial to develop effective analytical methods for their enantioselective discrimination and resolution during the development of related chiral drugs. In this context, chiral high performance liquid chromatography (HPLC) using chiral stationary phases (CSPs) have proven to be a powerful, effective, widely used and immensely valuable analytical tool for stereoselective discrimination and analysis of chiral compounds.[5,10-14] Polysaccharide derived CSPs have demonstrated high chiral recognition abilities and revolutionized the field of liquid chromatographic chiral separation.[5,12,14-16] Among them, amylose derived CSPs have shown effective recognition ability and broad applicability, successfully resolving a wide range of structurally diverse chiral compounds.[15-17] For the chromatographic discrimination of low UV absorption chiral amines on the selected CSP, derivatization of chiral amines with strong UV absorption reagents has been used to achieve improved enantioselectivity and higher detection sensitivity.[18,19] Previously, we have employed several aromatic derivatizing agents, including fluorene-2-carboxaldehyde, 9-anthrylaldehyde, and 4-chloro-7-nitro-1,2,3-benzoxadiazole chloride, for the enantiomeric discrimination of chiral amines using polysaccharide derived CSPs on normal phase HPLC.[5,14,20] We also reported the liquid chromatographic enantiomeric separation of chiral amines as naphthaldimine derivatives on several CSPs derived from polysaccharides, using three naphthaldehyde derivatizing agents, including 2-hydroxynaphthaldehyde.[21] In this current investigation, we have incorporated 2-hydroxynaphthaldehyde that can serve as potent derivatizing agent to augment the enantiomeric discrimination and resolution of three typical chiral amines (α-methylbenzylamine, 2-amino-4-methyl-1-pentanol, and 1-methylheptylamine) (*Fig.* 1) on several amylose trisphenylcarbamate derived CSPs. We expected that the aromatic 2-hydroxynaphthyl moiety of the derivatized analytes might interact with the chiral selector of the CSP for enantiomer separation, along with the enhanced detection of chiral amines.[5,21,22] Thus, in this study, we aim to develop a simple and convenient normal chiral HPLC method to resolve the enantiomers of chiral amines as 2-hydroxynaphthaldimine derivatives on six amylose trisphenylcarbamate derived CSPs under UV detection. Also, the chiral recognition mechanism involved in the enantiodiscrimination of 2-amino-4-methyl-1-pentanol with amylose tris(3,5-dimethylphenylcarbamate) chiral selector was elucidated through molecular docking study.[23-25] The involved key interactions and binding energies contributing to the chiral separation process of the investigated analyte were described and compared with the HPLC experiments results.

2. Experimental

2.1. Instrumentation

Enantiomer separation experiments of the three chiral amines were performed on an Agilent 1100 HPLC system (Palo Alto, CA, USA). The HPLC system was equipped with the following components: a G1322A vacuum degasser, a G1310A isocratic pump, a G1313A autosampler, a G1316A thermostatic column compartment, and a G1315A multiwavelength UV detector. The data was gathered using Hewlett-Packard (HP) ChemStation software. Six polysaccharide CSPs (four covalently bonded type and two coated type) derived from amylose trisphenylcarbamates, used for the entire enantioseparation, were sourced commercially. The four covalently bonded type CSPs, Chiralpak IA [amylose tris(3,5-dimethylphenylcarbamate)], Chiralpak ID [amylose tris(3-chlorophenylcarbamate)], Chiralpak IE [amylose tris(3,5-dichlorophenylcarbamate)], and Chiralpak IF [amylose tris(3-chloro-4-methylphenylcarbamate)] were obtained from Daicel Company (Tokyo, Japan). The other two

coated type CSPs, Chiralpak AD-H and Lux Amylose-1 [amylose tris(3,5-dimethylphenylcarbamate)], were procured from Daicel Company (Tokyo, Japan) and Phenomenex (Torrance, CA, USA), respectively. The dimension of all the used chiral columns was 250 mm × 4.6 mm, with a pore size of 5 μm.

2.2. Reagents and chemicals

HPLC grade hexane, 2-propanol, and ethanol, utilized for both the mobile phase and sample preparation, were obtained from Burdick & Jackson (Morristown, NJ, USA). Three chiral amine analytes of racemic 1-methylheptylamine; racemic, (R)- and (S)-2-amino-4-methyl-1-pentanol and α-methylbenzylamine (≥99 % purity), as well as magnesium sulfate, were purchased from either Sigma-Aldrich (St. Louis, MO, USA) or Alfa Aesar (Haverhill, MA, USA). The derivatizing agent, 2-hydroxynaphthaldehyde, was acquired from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Derivatization procedure and chromatographic conditions

The chiral amines as 2-hydroxynaphthaldimine derivatives were prepared by stirring each chiral amine, 2-hydroxynaphthaldehyde (1-2 equivalents) and excess magnesium sulfate (7 equivalents) in 2-propanol at room temperature for 6 h, according to the conventional method (*Fig.* 1).[26] In derivatization process, 2-propanol as a reaction solvent was used to ensure the safety of the coated type column and to maintain the integrity of the normal HPLC experiments. After then, the resulting mixture was filtered to eliminate excess magnesium sulfate, and the filtrate was further diluted to an appropriate concentration for direct injection into the normal chiral HPLC system. Each sample mixture underwent two injections to ensure the reproducibility and precision in the obtained results. Enantioseparation analysis of three chiral amines as 2-hydroxynaphthaldimine derivatives was carried out at a room temperature (25 °C) with a sample injection volume of 1 μL and the adjusted mobile phase flow rate of 1.0 mL/min under UV 310 nm detection. The mobile phase of 10 % 2-propanol/hexane (V/V) was used for the elution of investigated analytes as 2-hydroxynaphthaldimine derivatives on amylose derived CSPs. The employed mobile phase underwent filtration through a membrane filter (Millipore Corporation, Bedford, MA) with a pore size of 0.45 μm and was degassed by an ultrasonic bath (Branson, MI, USA) prior to use. Chromatographic parameters of retention factor (k), separation factor (α), and resolution (Rs) was also calculated.

2.4. Molecular docking simulation

Docking of the studied stereoisomers was done using an Intel® Pentium® Gold CPU (3.10 GHz) on Windows 10 education operating system. The ligands employed in this study comprised of (R)- and (S)-2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives. ChemBioDraw Ultra (12.0 version) was exploited to draw the derivatized enantiomeric structures of 2-amino-4-methyl-1-pentanol, which

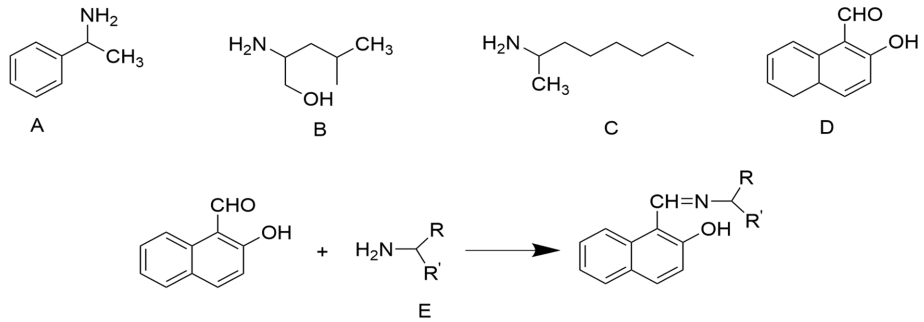


Fig. 1. Chemical structures of three typical chiral amines: (A) α-methylbenzylamine, (B) 2-amino-4-methyl-1-pentanol, and (C) 1-methylheptylamine. (D) shows 2-hydroxynaphthaldehyde derivatizing agent, while (E) illustrates the preparation of derivatized chiral amine analytes as 2-hydroxynaphthaldimine derivatives.

were then cleaned, optimized, converted to 3D, and saved in PDB format for use in the simulations. The receptor, amylose derived CSP with amylose tris(3,5-dimethylphenylcarbamate) as chiral selector, was acquired from prior research studies and was optimized to its minimum energy state before being saved in PDB format for simulations.[27,28] AutoDock 4.2.6 (Scripps Research Institute, La Jolla, CA, USA), and PyMOL (2.2 version) software was employed for the docking simulations to elucidate the interactions of the 2-amino-4-methyl-1-pentanol enantiomers with the amylose tris(3,5-dimethylphenylcarbamate) derived CSP.[29] AutoDock Tools (ADT) 1.5.6. (graphical interface) was employed to process the ligand and receptor structures.[30] This involved assigning Kollman and Gasteiger charges, merging nonpolar hydrogens, and saving the structures in PBBQT file format. The docking procedure allowed all the bonds of ligands as rotatable and receptor as rigid structure. The active 3D affinity grid was produced by employing AutoGrid program using the x, y, and z coordinates. The grid box of size 50 × 50 × 50 Å with a grid spacing of 0.375 Å was used for docking. The docking was accomplished using the Lamarckian genetic algorithm (LGA) to find the binding conformations of the flexible ligand to the receptor by setting the number of the genetic algorithm (GA) run to 100.[31] Obtained conformations of ligands with receptors were ranked using force field and empirical scoring function.[23,31] PyMOL was used to do recognition study by obtaining an image of binding interactions as well as the bond length of the hydrogen bonds between the receptor and enantiomers.[29] The best docked pose of studied analytes was selected based on its favorable interactions with receptor and the highest binding free energy observed.[32]

3. Results and Discussion

The enantioselective discrimination and resolution results for three chiral amines as 2-hydroxynaphthaldimine derivatives on amylose trisphenylcarbamate derived CSPs are summarized in *Table* 1. In this study, three typical chiral amines with distinct structural features were selected: an aromatic amine (α-methylbenzylamine), an aliphatic amine (1-methylheptylamine), and an aliphatic amino alcohol (2-amino-4-methyl-1-pentanol) characterized as hydroxylamine. The effects of the nature of three chiral amines and the structures of amylose chiral selectors on their chiral recognition ability were studied. Overall, good enan- tioseparation and resolution of the studied three chiral amines as 2-hydroxynaphthaldimine derivatives on six amylose derived CSPs was observed using normal chiral HPLC (*Table* 1). Especially, the enantioseparation efficiencies of two CSPs, Chiralpak ID and Chiralpak IF were higher than the other four amylose trisphenylcarbamate derived CSPs for investigated chiral amine analytes. As shown in *Table* 1, superior enantioselectivities of aromatic amine analyte (α-methylbenzylamine, *entry 1*) was achieved on amylose trisphenylcarbamate derived CSPs except for Chiralpak IE under the same analytical conditions. The highest enantioseparation and resolution was observed on Chiralpak ID for 2-hydroxynaphthaldimine derivative of α-methylbenzylamine amongst three analytes (*Table* 1, α = 1.63 and Rs = 6.44). For enhanced the enantioseparation of α-methylbenzylamine analyte, it is

Table 1. Enantiomeric separation of three chiral amines as 2-hydroxynaphthaldimine derivatives on six amylose trisphenylcarbamate derived CSPs

Entry	Analytes	Covalently bonded type CSPs												Coated type CSPs					
		Chiralpak IA			Chiralpak ID			Chiralpak IE			Chiralpak IF			Chiralpak AD-H			Lux Amylose-1		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1.	α-methylbenzylamine	1.45	3.63	6.97(R)[a]	1.63	4.15	6.44(R)	1.13	3.63	1.68(R)	1.34	2.19	4.59(R)	1.29	4.96	4.27(R)	1.34	4.84	6.03(R)
2.	2-amino-4-methyl-1-pentanol	1.14	1.93	1.70 (R)	1.20	3.99	2.76(R)	1.21	5.79	2.50(R)	1.23	3.25	2.95(R)	1.15	2.19	1.72(R)	1.16	2.78	2.25(R)
3.	1-methylheptylamine	1.08	2.85	1.28	1.59	6.91	3.89	1.08	7.00	1.04	1.20	5.14	3.16	1.05	3.44	0.62	1.04	3.78	0.64

Mobile phase: 10 % 2-propanol/hexane (V/V), Flow rate: 1 mL/min, Detection: UV 310 nm, α: Separation factor, k'_1: Retention factor of the first eluted enantiomer, Rs: Resolution factor, CSP: chiral stationary phase, [a]the absolute configuration of the second eluted enantiomer.

regarded that the aromatic moiety of α-methylbenzylamine could provide favorable interaction site with chiral selector of CSPs for enantiodiscrimination. For the other two aliphatic amine analytes, it is notable that better enantioseparation and resolution of amino alcohol analyte (2-amino-4-methyl-1-pentanol) (α = 1.14~1.23) was achieved than that of aliphatic amine (1-methylheptylamine) except Chiralpak ID in *Table* 1. It is considered that the hydroxyl group present in amino alcohol analyte (*Table* 1, *entry 2*) might be the crucial factor for the hydrogen bondings during the chiral interaction with the chiral selector of the CSP. The aliphatic amine analyte (*Table* 1, *entry 3*) was only partially enantioseparated (α = 1.04~1.08) on Chiralpak IA, Chiralpak IE, Chiralpak AD-H and Lux Amylose-1. However, as mentioned before, in this study, exceptionally good enantioseparation and resolution on Chiralpak ID derived from amylose tris(3-chlorophenylcarbamate) and/or Chiralpak IF amylose tris(3-chloro-4-methylphenylcarbamate) were observed. In general, substitution with either electron donating or electron withdrawing groups at the meta or para positions on the phenyl moieties can improve the chiral discrimination abilities of amylose phenylcarbamates.[16,33] In particular, amylose tris(3,5-dimethylphenylcarbamate) is a chiral selector of the powerful and widely used CSPs (Chiralpak AD-H and Lux Amylose-1) for the enhanced enantiomeric resolution of many racemic compounds including drugs.[16,33] The introduction of electron donating methyl groups helps maintain a more regular, rigid structure of CSPs through strong intramolecular hydrogen bondings, while electron withdrawing chloro groups augment the acidity of N-H groups in carbamate moieties.[16,33,34] In this study, the superior chiral discrimination ability showed by monochlorosubstituted Chiralpak ID (*Table* 1) might be the appropriate interactions of more acidic N-H groups with racemic analytes via strong hydrogen bondings.[33,34] Additionally, the greater enantioselectivities observed with Chiralpak IF, due to the disubstitution of both chloro and methyl groups at 3 and 4 positions, likely result from a synergistic balance of the aforementioned effects.[33,34] Regardless of the substitution effect on phenyl group of the above mentioned CSPs, it was worth noted that an identical elution order was observed for two analytes (α-methylbenzylamine and 2-amino-4-methyl-1-pentanol) as 2-hydroxynaphthaldimine derivatives on all amylose backbone CSPs in *Table* 1, with (R)-enantiomers being secondly eluted. Interestingly, in previous results, regardless of three naphthaldehyde derivatizing agents, consistent elution orders of all naphthaldimine derivatized analytes on amylose-derived CSPs were shown, as (R)-enantiomers being selectively eluted.[21] So, it is considered that the main chiral recognition mechanisms observed in this study are identical, even if the substituents on the phenyl group and CSP type of chiral selector used in this study influence their enantioselectivities to some extent. *Fig.* 2 depicts the typical HPLC chromatograms of α-methylbenzylamine and 2-amino-4-methyl-1-pentanol as 2-hydroxynapthaldimine derivatives on Chiralpak ID and Lux Amylose-1.

Molecular docking simulation technique was employed to explore the chiral discrimination process by providing the information on the binding energies and types of interactive forces involved in each

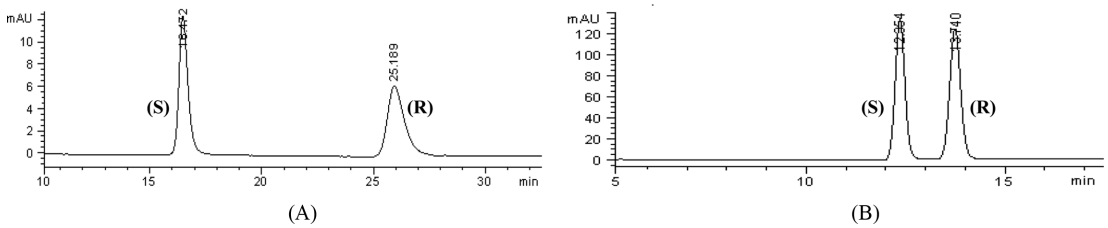
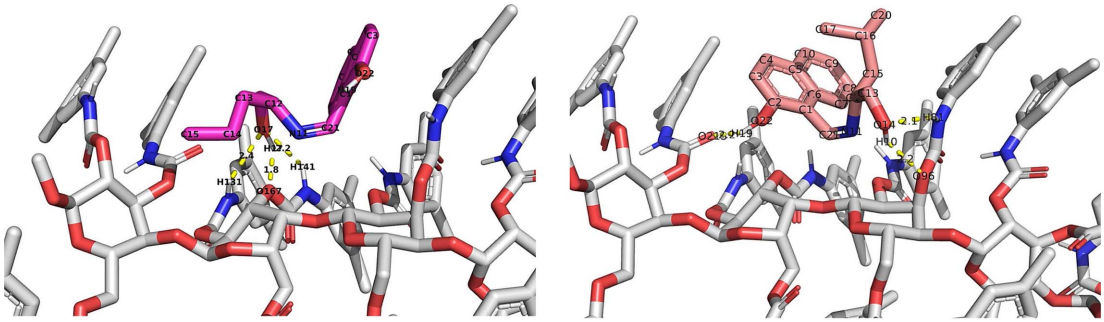


Fig. 2. Chiral HPLC chromatograms for the enantioselective discrimination of (A) α-methylbenzylamine and (B) 2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives on Chiralpak ID and Lux Amylose 1, respectively, Mobile phase: 10 % 2-propanol/hexane (V/V), Flow rate: 1 mL/min, Detection: UV 310 nm.

Table 2. Docking simulation results of (R)- and (S)-enantiomers of 2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives on amylose tris(3,5-dimethylphenylcarbamate) chiral selector with comparison to HPLC data on Chiralpak AD-H and Lux Amylose-1

Derivatized analyte	R/S enantiomer	Binding energy ΔE (kcal/mol)	Difference in binding energy ΔΔE (kcal/mol)	Number of hydrogen bondings	Distances of hydrogen bondings (Å) with OH moiety		Experimental data		
					Hydrogen bonding donor	Hydrogen bonding acceptor	Separation factor (α) Chiralpak AD-H/Lux Amylose-1[a]	Secondly eluted enantiomer	Difference in binding energy ΔΔG (kcal/mol)[b]
2-hydroxynaphthaldimine	(R)-isomer	-5.76	-0.14	3	1.8	2.2, 2.4	1.15/1.16	R	-0.08/-0.09
	(S)-isomer	-5.62		3	2.2, 2.2[c]	2.1			

[a]These experimental results obtained on two CSPs under the HPLC conditions [10 % 2-propanol/hexane (V/V), 1 mL/min, UV 310 nm], respectively, [b]The binding energy difference data calculated by the equation of ΔΔG = - RT ln α on two CSPs, respectively, [c]It is observed that there is another hydrogen bonding interaction between 2-hydroxy group on the derivatizing agent used for 2-hydroxynaphthaldimine derivative and carbamate ester oxygen on chiral selector of CSP [*Fig.* 3(B)].



(A) (B)

Fig. 3. 3D stereospecifically fit and stable inclusion complexes of enantiomers of (A) (R)- and (B) (S)-2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives with amylose tris(3,5-dimethylphenylcarbamate) chiral selector.

enantiomer (guest)-chiral selector (host) complex at the supramolecular level.[23-25,32,35] We investigated the stereodependent binding interactions and affinities of the two enantiomers of 2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives on the most widely used amylose tris(3,5-dimethylphenylcarbamate) chiral selector derived CSP (Chiralpak AD-H or Lux Amylose-1). In highly order helical structure of amylose tris(3,5-dimethylphenylcarbamate) chiral selector, enantiodiscrimination occurs if the ligand (each enantiomer) better fit into the chiral grooves of the selector and the fitting of the enantiomers were stabilized by diverse interactive forces.[23,27,35-37] *Fig.* 3 and *Table* 2 show the stereospecifically fitted, most stable three-dimensional analytes-CSP inclusion complexes and modeling data for the two enantiomers of 2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives with amylose tris(3,5-dimethylphenylcarbamate) chiral selector. The binding affinities of (R)- and (S)-2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives were -5.76 and -5.62 kcal/mole, respectively (*Table* 2). Clearly, the binding energies (ΔE) were negative for the derivatized analytes, indicating that the formations of the analyte-CSP complexes were enthalpy driven and occurred spontaneously.[32,35] More negative values of ΔE correspond to greater stability of enantiomer-CSP binding complex.[32,37,38] From *Fig.* 3, the primary binding interaction of the active enantiomer on the active site of CSP, which contributed to enantiodiscrimination, can be seen. As observed, the main interactive forces involved between CSP and analytes for the formation of stable complexes are considered to be intermolecular hydrogen bonding interactions. In case of (R)- and (S)-2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives, as depicted in *Fig.* 3(A) and 3(B), three intermolecular hydrogen bonding interactions in both cases between hydroxyl

moieties of each derivatized enantiomer and C = O, N-H, and ester oxygen of the carbamate of the chiral selector were observed. Detailed information about H-donor and H-acceptor for the observed hydrogen bonding interactions is shown in *Table* 2. Among several hydrogen bonding donor or acceptor interactions, specifically, it was observed that the first hydrogen bonding interaction formed between hydroxyl group present in the (R)-amino alcohol analyte as 2-hydroxynaphthaldimine derivative and the carbamate ester oxygen of the chiral selector (CSP), was the strongest [1.8 Å bond length in *Fig.* 3(A)]. Shorter hydrogen bonding distances (Å) between the enantiomers with CSP imply the higher affinity of the enantiomer-CSP complexes.[37] This interaction appeared to be vital for the contributing to the stable complex formation and higher binding energy of (R)-enantiomer as 2-hydroxynaphthaldimine derivative (-5.76 kcal/mol, *Table* 2). In molecular docking simulation, the elution order and the enantioselectivity (α) of a pair of enantiomers can be predicted and rationalized by considering the binding affinities and their differences (ΔE and ΔΔE, kcal/mol).[27,32,36] *Table* 2 shows the comparative experimental (HPLC) and theoretical findings of 2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivative on amylose tris(3,5-dimethylphenylcarbamate) derived CSP (Chiralpak AD-H and Lux Amylose-1). As shown in *Fig.* 2(B) of HPLC data, the secondly eluted enantiomer on tris(3,5-dimethylphenylcarbamate) derived CSP was (R)-2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivative. As illustrated, the binding energies of (R)- and (S)-enantiomers of 2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives, exhibited the following order: -5.76 kcal/mol for the (R)-enantiomer, and -5.62 kcal/mol for the (S)-enantiomer, respectively. Thus, from the observed interactions, the docking simulation prediction Top of Formfor the second eluted enantiomer was also (R)-enantiomer, which clearly supported the order of elution of the enantiomers of 2-amino-4-methyl-1-pentanol in the chromatographic data. The difference in binding energies ($\Delta\Delta E_{R-S}$) between a pair of enantiomers and CSP in docking simulations can be related to separation factor (α) obtained from chromatographic data. From *Table* 2, the binding energy difference (ΔΔE) found between (R)- and (S)-2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives for theoretical approach was found to be -0.14 kcal/mol. The observed findings showed agreement with the experimental data [ΔΔG = -0.08, -0.09; α = 1.15, 1.16 in *Table* 2 for ΔΔG = -RT ln α; separation factor (α) in HPLC]. Predictions from molecular docking study could offer significant information about chiral recognition.[23,32] This approach enables the screening of the potential chiral selectors, aiding in the design of experiments aimed at enhancing the resolution of chiral compounds.[32]

4. Conclusions

In summary, a convenient and reproducible chiral HPLC method was developed to separate the enantiomers of three typical chiral amines as 2-hydroxynaphthaldimine derivatives using six amylose trisphenylcarbamates derived CSPs with a normal phase eluent. The amylose trisphenylcarbamates chiral selectors were found to be highly effective for the successful enantioselective discrimination and resolution of three structurally different chiral amines by chiral HPLC. Especially, CSPs based on monosubstituted halogen or a combination of alkyl and halogen group chiral selectors [amylose tris(3-chlorophenylcarbamate) and amylose tris(3-chloro-4-methylphenylcarbamate)] showed higher enantiorecognition abilities in discriminating the enantiomers of three studied chiral amines. The presence of aromatic or hydroxyl moiety of chiral amines in this study showed the positive impact on chiral interaction with chiral selectors for the selectively effective and enhanced enantioseparation of α-methylbenzylamine and 2-amino-4-methyl-1-pentanol analytes. Based upon molecular modeling study for chiral recognition mechanism between 2-amino-4-methyl-1-pentanol as 2-hydroxynaphthaldimine derivatives and amylose tris(3,5-dimethylphenylcarbamate) chiral selector of the most widely used CSP, we described the chiral recognition interactions with the elution order which support the observed chiral

HPLC experiments. It was observed that intermolecular hydrogen bonding interactions were the main forces involved for the enantioselective analyte-CSP inclusion complexes. It is expected that this docking simulation study could be a very useful tool for designing chiral selectors and optimizing chiral HPLC experiments obtained using different derivatizing agents. Also, from theoretical approaches, it is easy to predict elution order related to chiral recognition before conducting the experiments.

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