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Preparation and evaluation of a triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase for HPLC

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Abstract

A triazole-bridged *bis*(β-cyclodextrin) was synthesized via a high-yield *Click Chemistry* reaction between 6-azido-β-cyclodextrin and 6-propynylamino-β-cyclodextrin, and then it was bonded onto ordered silica gel SBA-15 to obtain a novel triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase (TBCDP). The structures of the bridged cyclodextrin and TBCDP were characterized by the infrared spectroscopy, mass spectrometry, elemental analysis, and thermogravimetric analysis. The chiral performance of TBCDP was evaluated by using chiral pesticides and drugs as probes including triazoles, flavanones, dansyl amino acids and β-blockers. Some effects of the composition in mobile phase and pH value on the enantioseparations were investigated in different modes. The nine triazoles, eight flavanones, and eight dansyl amino acids were successfully resolved on TBCDP under the reversed phase with the resolutions of hexaconazole, 2′-hydroxyflavanone, and dansyl-*DL*-tyrosine, which were 2.49, 5.40, and 3.25 within 30 minutes, respectively. The ten β-blockers were also separated under the polar organic mode with the resolution of arotinolol reached 1.71. Some related separation mechanisms were discussed preliminary. Compared with the native cyclodextrin stationary phase (CDSP), TBCDP has higher enantioselectivity to separate more analytes, which benefited from the synergistic inclusion ability of the two adjacent cavities and bridging linker of TBCDP, thereby enabling it a promising prospect in chiral drugs and food analysis.

KEYWORDS

bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase, chiral drugs and pesticides, enantiomeric separations, high-performance liquid chromatography, preparation and evaluation

1 | INTRODUCTION

Chiral compounds have been widely used in medical treatment and agricultural production due to their high biological activities.[1-4] Many studies have confirmed that enantiomers of chiral drugs and pesticides have different properties in biological activities and pharmacokinetics, with usually only one of them has desired effects,[1] and the others are not only ineffective but also harmful to the human bodies. For example, *R*-propranolol is 100 times weaker than *S*-propranolol in antihypertensive effect and even may cause the male infertility.[2] At present, most of chiral compounds still have been using in the racemic form due to difficulty in synthesis and purification, which made the enantiomeric separation thriving.[3,4] With effective resolution and universal

applicability, the high-performance liquid chromatography (HPLC) coupling various chiral stationary phases (CSPs) has developed into one of the most important methods in the chiral separation, in which the efficient and versatile CSPs has played a crucial role in this method.[5]

The most common CSPs derivatized the cellulose-based stationary phase, of which the separation capability mainly depends upon the effects of inclusion, hydrogen bonding, and ion-association provided by the ordered grooves of the polysaccharides.[6] For the purpose of protecting the ordered structure, this type of CSPs is usually used in a coating manner, with being employed under the normal phase (NP) mode that uses the non-aqueous hexane-isopropanol as the mobile phase.[7,8] Therefore, it is relatively insufficient for these cellulose-based CSPs in the compatibility of being combined with electrospray mass spectrometry (ESI-MS). As we know, the exploration of the multimode CSPs (MMCSP) has an important significance.[9,10] The cyclodextrin-based CSPs (CD-CSPs),[11] characterized by the multimode compatibility, which is applicable to the reversed phase (RP),[12] NP,[13] and polar organic (PO) mode,[14] have been frequently used in HPLC.

In 1985, Armstrong[15] reported the first stable β-CD-CSP and applied a patent. After that, most studies have focused on the functionalization of CD-CSPs such as alkylation, benzoylation, and phenylcarbamated,[16-18] which introduced various interaction sites including π-π stacking, dipole-dipole, ion paring, electrostatic attraction, and steric repulsive for the chiral separation. However, the performance of CD-CSPs could be influenced by the number of substituents on the CD's rims.[19] By far, according to the degree of substitution, the current derivatization of CD-CSPs has been divided into *mono*derivatization, partial derivatization, and full derivatization. The *mono*derivatized CD-CSPs has well-defined structures and chromatographic stability, but the single substituent may be inefficient to improve the enantioselectivity.[20] The partial-derivatized CD-CSPs has suitable substituents, but with incapability to ensure the number and the position of substituents that caused the poor chromatographic reproducibility. The full-derivatized CD-CSPs could readily be obtained by adding excess modifiers. Armstrong research group[12,14,17,18] has prepared a series of fully aromatic-derivatized CD-CSPs and systematically investigated the chiral separation mechanism of them. They separated a large spectrum of enantiomers including the derivatized chiral amines, alcohols, and amino acids. However, the dansyl amino acids, crown ethers, and some biologically active compounds were not resolved on their phenethyl- and naphthylethyl-carbamate fully derivatized CD-CSPs.[17] They attributed this to the space steric hindrance caused by the excessive substituents, leading to the congestion of the CD's rims and incurring difficulty for the CD's cavities to include the analytes.[19] Therefore, only relying on the functionalization is limited in improving the chiral performance of the CD-CSPs. A promising alternative is developing the novel bridged CD-CSPs.

The bridged cyclodextrins is a new kind of supramolecular compounds coupled by two or more native cyclodextrins.[21] By bridging two or more cavities with a functional linker, the bridged cyclodextrins could significantly enhance the original binding ability and molecular selectivity of the native cyclodextrin, thus being extensively used in molecular recognition, asymmetric catalysis, drug-directed carriers, and artificial mimic enzymes.[22-24] Many studies have confirmed that the binding constant of the bridged cyclodextrins with guests was as high as the level of biological enzymes.[23] Moreover, Liu et al[24] found that in addition to the synergistic inclusion, the bridging functional linker could also act as a *pseudo-cavity*[21] to generate extraordinary effects. Although the bridged cyclodextrins has attracted great attention for a long time, few reports can be found in chiral separation. Chang et al[25] prepared three diamino-bridged *bis*(β-CD)-CSPs and separated different sets of chiral compounds including aryl alcohols and β-blockers, and acquired the encouraging resolutions. Ai et al[26] prepared two ureido-bridged β-CD CSPs and achieved the good resolutions for aromatic positional isomers and dansyl amino acid enantiomers, which supported the synergistic inclusion of bridged cyclodextrins in separations. Our research group[27] also prepared an ethylenediamino-bridged *bis*(β-CD)-CSP to resolved fourteen β-blocker drugs, of which exhibited higher resolutions and wider enantioselectivities than the native CD-CSP. Obviously, the bridged CD-CSPs has a potential applicable value for chiral separations.

The bridged *bis*(β-cyclodextrin) is usually synthesized by the bifunctional reagents such as diisocyanates, dicarboxylic acids, and diacid chlorides.[22,25,28] But the steric hindrance and excessive reaction sites (–OH) of the bulky cyclodextrins would lead to the above-mentioned synthetic approaches inclining a lower yield (5-15%), thus having to purify by time-consuming and complicated process.[22] A satisfactory alternative is the *Click Chemistry* reaction proposed by Sharpless for the first time.[29] As a powerful, highly reliable and selective reaction to rapidly synthesize various new compounds, the [3 + 2] dipolar cycloaddition between azides/alkynes catalyzed by copper (I) is a primary *Click Chemistry* reaction.[30]

We herein described a new approach to prepare the triazole-bridged *bis*(β-CD)–bonded CSP (TBCDP) via

Click Chemistry reaction between 6-azido-β-cyclodextrin (N_3-CD) and 6-propynylamino-β-CD (PA-β-CD) and then bonding the resulted bridged cyclodexrins onto the surface of SBA-15. After chemical structure characterization, the chiral performance of TBCDP was systematically evaluated by using different racemic drugs and pesticides as analytes in the RP and PO modes. Some chromatographic conditions such as mobile phase composition, pH values, and column temperature on the enantioselectivity were optimized, and some related chiral separation mechanisms of the TBCDP were preliminarily discussed.

2 | MATERIALS AND METHODS

2.1 | Materials and instruments

Triblock polymers P 123 (MW ~5800), tetraethyl orthosilicate (TEOS), 1,3,5-trimethyl-benzene (TMB), β-cyclodextrin (β-CD), and 3-isocyanatopropyltriethoxysilane were purchased from Sigma-Aldrich. *p*-Toluenesulfonyl chloride (*p*-TsCl), propargylamine, triphenylphosphine, sodium azide, and cuprous iodide (CuI) were analytical grade and purchased from Aladdin-Reagent (Shanghai, China). Racemic standards (flavanones, amino acids, and β-blockers) were purchased from Sigma-Aldrich. Triazole racemic standards (purity ≥ 98%) were purchased from Shanghai Pesticide Research Institute (Shanghai, China). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were purchased from Tedia Company (Inc, USA). Formic acid (FA), acetic acid (HOAc), triethylamine (TEA), *N*,*N*-dimethylformamide (DMF), acetone, and other chemicals were all analytical reagents purchased from the Sinopharm Chemical Reagent Co, Ltd (Shanghai, China). DMF was treated with CaH_2 to remove water before use.

All HPLC chiral separations were performed at a ZQ4000/2695 LC/MS equipped with 2966 diode array detector (Waters, USA). Infrared characterization was performed on a Nicolet 5700 Fourier-transform infrared spectrometer (Thermo, USA). Elemental analysis was performed on a Vorio EL III (Elementar, Germany). Thermogravimetric analysis was performed on a Diamond TG/DTA (Perkin, Elmer, USA). The HPLC column was packed by an AW-60 chromatographic packing device (Haskel, USA). Ultra-pure water (resistivity >18.2 MΩ.cm) was prepared by a Milli-Q water purification system (Millipore, USA).

2.2 | Preparation of triazole-bridged *bis*(β-CD)-CSP

The synthetic route of TBCDP was shown in Figure 1, including the preparation of triazole-bridged *bis*(β-CD) and its immobilization.

2.2.1 | Preparation of SBA-15

According to the improved method of Zhao et al,[31] a spherical and ordered mesoporous materials of SBA-15 was prepared by our group[16] and used as the silica gel matrix for TBCDP, which has the particle diameter of

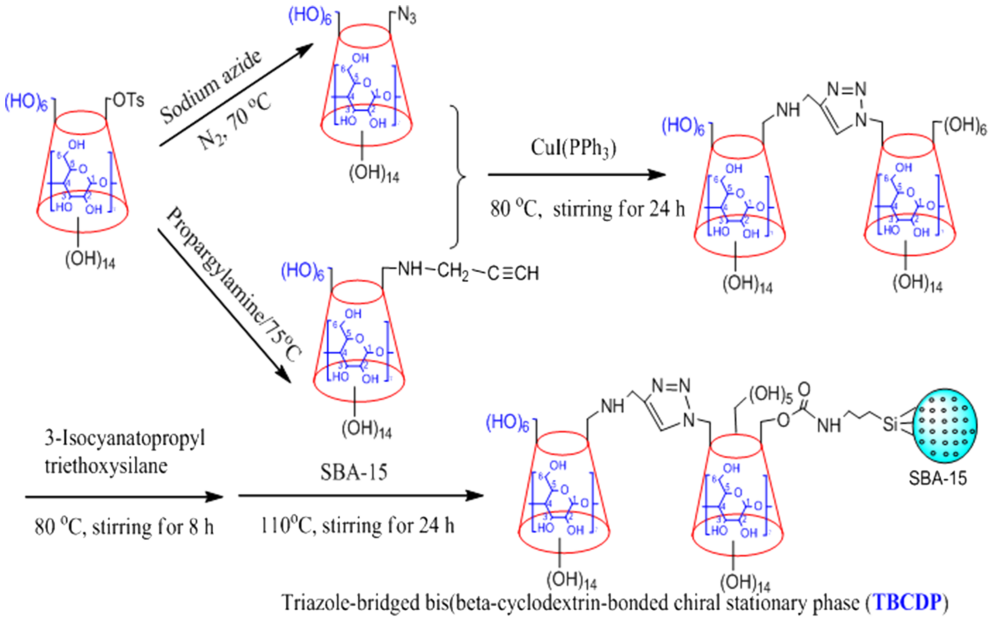

FIGURE 1 Synthetic route of triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase (TBCDP)

about 3.2 μm and the pore size of about 24 nm with the specific surface area of about 400 m^2/g. The procedure was briefly described as follows: 5.6-g P123 (template) and 12-g TEOS (silicon source) dissolved into 250 mL of 2.0 mol/L of HCl, after adding of 6.0-g TMB (porogen) and appropriate amount KCl, stirred at 38°C for 24 hours. Then the mixture was transferred to a hydrothermal autoclave and reacted statically at 110°C for 48 hours, and white solid was collected and calcined at 550°C for 10 hours to obtain the SBA-15.

2.2.2 | Synthesis of triazole-bridged *bis*(β-CD)

Triazole-bridged *bis*(β-CD) was synthesized by the *Click Chemistry* reaction. *Mono*-6-*deoxy*-6-azido-β-cyclodextrin (N_3-CD) and *mono*-6-*deoxy*-6-propynylamino-β-cyclodextrin (PA-CD) were synthesized according to the previous reports,[20,30] respectively. Starting from the readily obtained *mono*-6-(*p*-tosylsulfonyl)-β-CD (TsO-CD).

To a solution of TsO-CD (5.0 g, 3.9 mmol) in 50 mL of water was added sodium azide (1.26 g, 19.4 mmol). The mixture was stirred at 70°C for 24 hours under the atmosphere of nitrogen. After filtration, the filtrate was poured into 200-mL acetone. Then the obtained white precipitate was collected and washed with acetone for several times and dried at 50°C to obtain the product N_3-CD with a yield of 92.8%. ESI-MS: *m/z*, $[M + Na]^+$ calculated for 1183.01 and found at 1182.94 shown in Figure 2A.

TsO-CD (7.0 g, 5.4 mmol) was added to a 50-mL round bottom flask, and then 15-mL propargylamine was added dropwise. The mixture was stirred at 75°C for 20 hours before poured into 200-mL acetone. After filtration, the yellow precipitate was recrystallized twice from methanol and dried at 40°C to give the product of PA-β-CD with a yield of about 88.6%. ESI-MS: *m/z*, $[M + H]^+$ was calculated for 1172.08 and found at 1171.60 shown in Figure 2B.

The triazole-bridged *bis*(β-CD) was synthesis by the *Click Chemistry* reaction of the above resulted N_3-CD and PA-CD. To a solution of PA-CD (2.3 g, 19.6 mmol) in 50 mL of DMF was added CuI (PPh_3)[20] (0.025 g, 0.06 mmol) and N_3-CD (2.3 g, 19.8 mmol). After stirring at 80°C for 24 hours, the solvent was removed under a reduced pressure and then precipitated by adding acetone. The obtained precipitates were filtered and washed with acetone to give the triazole-bridged *bis*(β-CD) ligand. After dried at 50°C, the yield was calculated for about 62.0%. ESI-MS $[M + H]^+$ was calculated for 2332.07 and found at 2331.77 shown in Figure 2C.

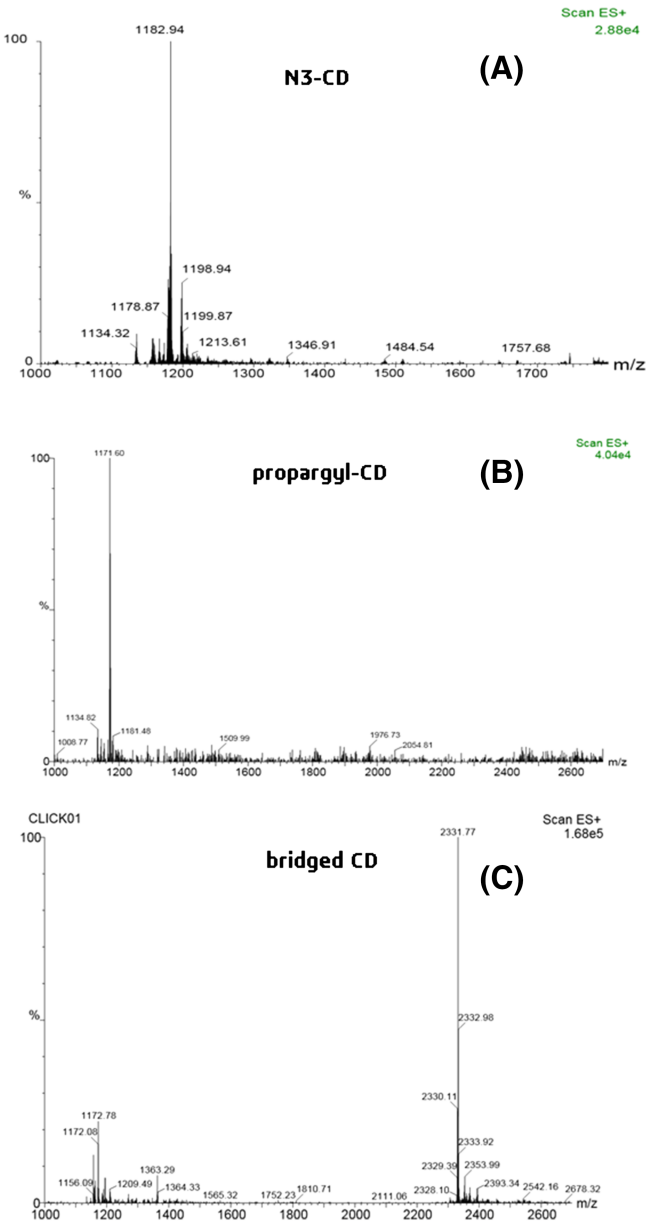

FIGURE 2 The electrospray mass spectrometry (ESI-MS) spectra of azido-β-cyclodextrin A propargylated β-cyclodextrin B and bridged *bis*(β-cyclodextrin) C

2.2.3 | Preparation of TBCDP

TBCDP was prepared by immobilizing the above synthesized triazole-bridged *bis*(β-CD) onto the silica gel (SBA-15). To a solution of triazole bridged β-CD (1.5 g) in 40 mL of DMF was added 0.1 mL 3-isocyanatepropyltriethoxysilane by dropwise. The mixture was stirred at 80°C for 3 hours under the atmosphere of nitrogen. After adding of 3.0-g SBA-15 and reacted with stirring at 110°C for 24 hours. The mixture was filtered and washed repeatedly by DMF, MeOH, and acetone to give the final TBCDP.

The cyclodextrin stationary phase (CDSP) was prepared by the similar method of TBCDP. To a solution of β-CD (0.8 g) in 40 mL of DMF was added 0.3 mL 3-isocyanatepropyltriethoxysilane by dropwise. Then the 3.0 g of SBA as silica gel support was added. The rest of preparation steps was similar to of the TBCDP. The native cyclodextrin-bonded phase (CDSP) was obtained for the following comparative studies.

2.2.4 | Packing of the columns

The above-resulted TBCDP (3.5 g) and CDSP were packed into HPLC column in the conventional slurry approach, where methanol (30 mL) served as the packing solvent. The CSPs of TBCDP and CDSP were respectively suspended in acetone by ultrasonicating for 20 minutes and then packed into stainless steel columns (4.6 mm × 150 mm) under the constant pressure (about 34.5 MPa) maintained for 40 minutes by the packing system (Haskel, USA). Finally, the packing pressure was released to ambient gradually.

2.3 | HPLC procedures

2.3.1 | HPLC conditions

The mobile phases were prepared by mixing appropriate amounts of acetonitrile (ACN) or methanol (MeOH) into water. For the separations of some specific analytes, the mobile phases were revised by employing 1% (*v*/*v*) triethylammonium acetate buffer (1% TEAA, pH was adjusted by acetic acid) or 0.1% (*v*/*v*) FA (0.1% FA) in place of water. All analytes were dissolved in methanol to prepare stock solutions with the concentration of 100 to 200 μg/mL and stored at 4°C. Both of the mobile phases and stock solutions were filtered with 0.22-μm membrane and ultrasonicated for 30 minutes before use. The above stock solutions were diluted to appropriate concentration accordingly occasionally. The flow rate of mobile phase was 0.8 mL/min, the column temperature was selected at 20°C, which was easy to be controlled. According to the spectral properties of the analytes, the detection wavelength range was set 200 to 380 nm (flavanones at 280 nm, dansyl amino acids at 254 nm, and triazole pesticides at 220 nm). The injection volume was adjusted as 3 to 10 μL.

2.3.2 | HPLC evaluation parameters

Chromatographic performance of TBCDP was evaluated by following parameters (according to USP standards): The retention factor (k') calculated by the fomular of $k' = (t_R - t_0)/t_0$, respectively, where t_0 is the retention time at which the first baseline disturbance by the solvent peak appeared and t_R is the retention time of the each enantiomer. The separation factor (α) calculated according to the formula of $\alpha = k'_2/k'_1$, where k'_1 and k'_2 are the retention factor of the first and second enantiomer, respectively. The chiral resolution (*R*s) evaluated by the equation of $Rs = 1.18(t_{R2} - t_{R1})/(w_{h1} + w_{h2})$, where t_{R1} and t_{R2} are the retention time of the first and second enantiomers and w_{h1} and w_{h2} are the half-peak width of each enantiomer, respectively.

3 | RESULTS AND DISCUSSION

3.1 | Characterization of TBCDP

The prepared TBCDP was characterized by infrared spectroscopy, elemental analysis, and thermogravimetric analysis.

The infrared spectra of the bridged β-CD ligand and TBCDP were shown in Figure 3, where the representative peaks of cyclodextrin at ν_{O-H} (3365 cm^{-1}), ν_{C-H} (2926 and 2869 cm^{-1}), and ν_{C-O} (1160, 1082, and 1030 cm^{-1}) could be observed. The peak cluster of 1660, 1595, and 1465 cm^{-1} were attributed to $\nu_{C=N}$, $\nu_{N=N}$, and ν_{C-N} stretching vibration, which indicated that the triazole bridging linker was attached on the β-CD that implied the successful synthesis of the bridged β-CD ligand.

The infrared spectra of TBCDP were similar to ligand, with the characteristic peaks of triazole group at 1629.34 and 1543.51 cm^{-1}, indicating that the bridged β-CD ligand was bonded onto the silica gel. Successful immobilization of triazole-bridged *bis*(β-cyclodextrin) on SBA-15 was evidenced by the following adsorption peaks: 3341.63 cm^{-1} (ν_{O-H} of β-CD and silica gel) and 2933.48 cm^{-1} (ν_{C-H} of β-CD), which indicated that the organic layer was contained on silica gel. The characteristic absorption peaks of $\nu_{N=N=N}$ at 2124 cm^{-1} and ν_{C-H} and ν_{C-C} at 3249 and 2112 cm^{-1} almost disappeared, hinting that both of N═N═N and C≡C groups were exhausted by reacting with each other. The new peaks at 1629.34 and 1543.51 cm^{-1} respectively belong to the vibration of the C=N and triazole ring, indicating that the azido groups reacted with the terminal-alkynes via the "Click Chemistry" reaction. The peak cluster at 1237.00, 1155.26, 1102.14, and 1032.66 cm^{-1} were the typical absorptionsν_{C-O} of the β-CD and $\nu_{Si-O-Si}$ of the SBA-15.

The elemental analysis results were shown in Table S1. According to the carbon content (6.02%), the average loading of TBCDP was about 0.14 μmol/m^2 calculated by the formula: $C\%/(12 \times Nc \times S) \times 10^6$, where *N*c is

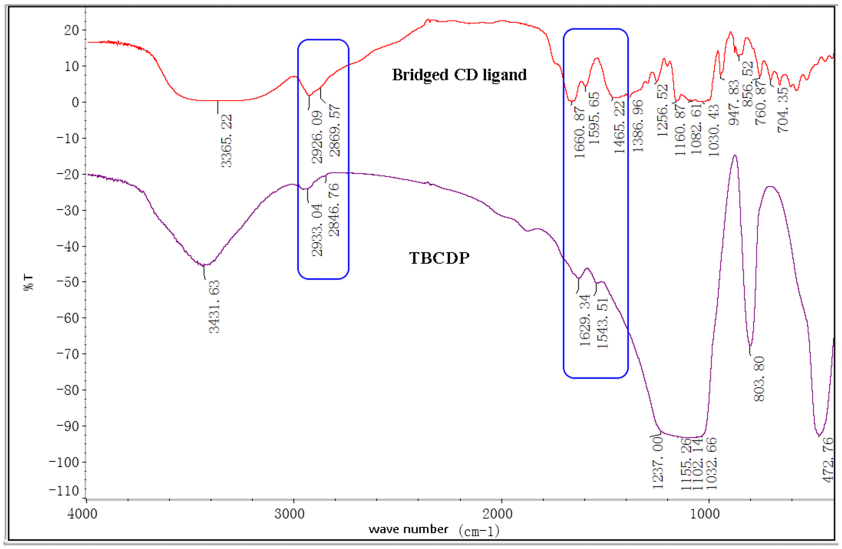

FIGURE 3 The FTIR spectra of bridged *bis*(β-cyclodextrin) and triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase (TBCDP)

the number of carbon atoms in per ligand molecule and S is the surface area of SBA-15 (400 m^2/g).

Likewise, according to the carbon content (6.18%), the average loading of CDSP was about 0.28 $\mu mol/m^2$.

The weight loss of TBCDP was determined by the thermogravimetric analysis in the range of recording temperature from 50°C to 900°C (10°C/min as the heating rate), and the weight loss result was 10.5% (*w*/*w*). The average loading of TBCDP was estimated to be 0.11 $\mu mol/m^2$ according to the weight loss result, which was corresponding to the loading result calculated on the basis of elemental analysis. Moreover, the excellent chiral chromatographic properties of TBCDP also evidenced the feasibility of the above proposed preparation method. In addition to chiral separations, TBCDP has also excellent selectivity to positional isomers (Figure S1).

3.2 | Chiral performance of TBCDP

3.2.1 | Chiral separation of triazole pesticides

The triazole chiral pesticide is one of the most widely used high-efficiency fungicides and plant growth regulators in the world. At present, more than 30 varieties of triazoles have been used in agricultural production and the preservation of fruits and vegetables. Since enantiomers of each chiral triazole pesticide has big difference in biological activity, toxicity, and residue behavior in animals and plants, the triazole pesticides in the United States have been classified as a potential chirality carcinogens requiring high monitor. Therefore, we choose common nine triazole pesticides (Figure 4) as chiral probes to evaluate the chromatographic performance of TBCDP. The corresponding results and chromatographic

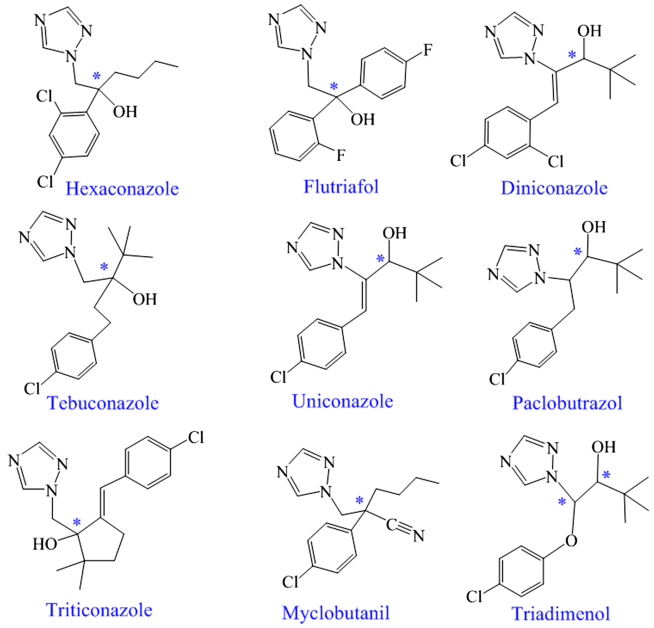

FIGURE 4 The chemical structures of nine triazole pesticides

conditions were shown in Table 1, as well as some chromatograms in Figure 5.

Both the TBCDP and CDSP were not derivatized; the inclusion effects of them should be responsible for their separation abilities in the RP chromatography.[14,17,18] In this experiment, we used simple methanol- and acetonitrile-water as mobile phase to separate the selected triazole pesticides under the RP mode. As shown in Table 1, nine triazoles were successfully resolved on the TBCDP with the resolutions (*R*s) from 0.50 to 2.49 within 30 minutes (Figure 5). The highest enantioresolution (*R*s = 2.49) for hexaconazole was due to the minimal steric hindrance near its chiral carbon, making the anayte convenient to enter or approach to the cavity of the cyclodextrin. However, CDSP only

TABLE 1 The enantioseparation results of triazole pesticides and better chromatographic conditions by HPLC

Compounds	k'_1	k'_2	α	*R*s	Mobile Phases	Columns
Hexaconazole	2.93	3.55	1.21	2.49	MeOH/H_2O (40/60,*v/v*)	TBCDP
	4.31	5.02	1.16	1.95	ACN/H_2O (20/80, *v/v*)	TBCDP
	3.56	4.17	1.17	1.18	MeOH/H_2O (40/60,*v/v*)	CDSP
Flutriafol	3.54	3.94	1.11	1.37	MeOH/H_2O (30/70, *v/v*)	TBCDP
	5.26	5.85	1.11	1.61	MeOH/ACN/H_2O (15/5/80, *v/v/v*)	TBCDP
	5.01	5.41	1.08	1.27	MeOH/H_2O (30/70, *v/v*)	CDSP
Diniconazole	6.24	6.81	1.09	1.12	MeOH/H_2O (35/65, *v/v*)	TBCDP
	6.68	7.34	1.10	1.42	MeOH/ACN/H_2O (25/5/70, *v/v/v*)	TBCDP
	7.08	7.58	1.07	1.25	MeOH/ACN/H_2O (25/5/70, *v/v/v*)	CDSP
Tebuconazole	5.97	6.35	1.06	0.65	MeOH/H_2O (40/60, *v/v*)	TBCDP
	6.81	7.27	1.07	1.19	ACN/H_2O (20/80, *v/v*)	TBCDP
	7.52	8.20	1.09	1.00	ACN/H_2O (20/80, *v/v*)	CDSP
Uniconazole	4.40	4.68	1.06	0.89	MeOH/H_2O (40/60, *v/v*)	TBCDP
	7.45	7.90	1.06	1.23	MeOH/ACN/H_2O (25/5/70, *v/v/v*)	TBCDP
	7.89	8.21	1.04	0.98	MeOH/ACN/H_2O (25/5/70, *v/v/v*)	CDSP
Paclobutrazol	6.54	7.70	1.18	1.15	MeOH/H_2O (40/60, *v/v*)	TBCDP
	4.56	5.21	1.14	1.28	ACN/H_2O (30/70, *v/v*)	TBCDP
	5.32	6.01	1.13	0.93	ACN/H_2O (30/70, *v/v*)	CDSP
Triticonazole	7.04	7.50	1.07	1.14	MeOH/H_2O (40/60, *v/v*)	TBCDP
	5.33	5.53	1.04	0.53	MeOH/H_2O (40/60, *v/v*)	CDSP
Myclobutanil	3.22	3.29	1.02	<0.5	MeOH/ACN/H_2O (25/5/70, *v/v/v*)	TBCDP
	2.89	/	/	0	MeOH/ACN/H_2O (25/5/70, *v/v/v*)	CDSP
Triadimenol	4.01	4.20	1.05	0.75	MeOH/H_2O (35/65, *v/v*)	TBCDP
	5.27	/	/	0	MeOH/H_2O (35/65, *v/v*)	CDSP

Abbreviations: CDSP, cyclodextrin stationary phase; HPLC, high-performance liquid chromatography; TBCDP, triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase.

separated seven types and with lower resolutions (*R*s, 0.53-1.28). This indicated that the two stationary phases were different in their chiral recognition under the RP mode. CDSP relies on a single cavity with small size (0.65 nm) that only benzene ring of the analytes was encapsulated by it so that most of solute molecules were exposed to the achiral mobile phase, thereby the chiral recognition of CDSP was insufficient. The two cavities of TBCDP integrated with bridging group, and the whole molecule of analytes colud be embedded in the bridged cyclodextrins, which could more accurately recognize the *R*- and *S*-configurations of analytes, resulting the separation capacity was broadened and enantioselectivity was improved, which was consistent with the synergistic inclusion confirmed by other means by Liu et al.[21,24,28]

In addition to inclusion, the hydrogen bonding is also important in chiral recognition. For example, seven triazoles containing –OH on their chiral carbons (Figure 4) had relatively high enantioresolutions. It was indicated that the hydrogen bonding between the –OH of the chiral carbon and the portal –OH of the cyclodextrins could contribute to chiral recognition.[13] Taking hexaconazole (*R*s = 2.49) and myclobutan (*R*s < 0.5) as an example, when the –OH was replaced by a cyano group (–CN), the resolution greatly decreased.

TBCDP has an advantage in separating the selected triazole pesticides only with using simple mobile phase composited by methanol or acetonitrile-water. From Figure S2, it could be seen the selected triazole analytes had stronger retention on TBCDP (about 30 min eluted by 30%-40% (*v/v*) of methanol in mobile phases). We found that by using high content of methanol mobile, the triazoles were eluted with faster speed companied with the decreasing of resolutions. In contrast, by using low content of methanol, the retention times of analytes were extremely prolonged leading to the broadening and asymmetrical peaks that was also unfavorable to separation. Considering that methanol was a protic solvent, which

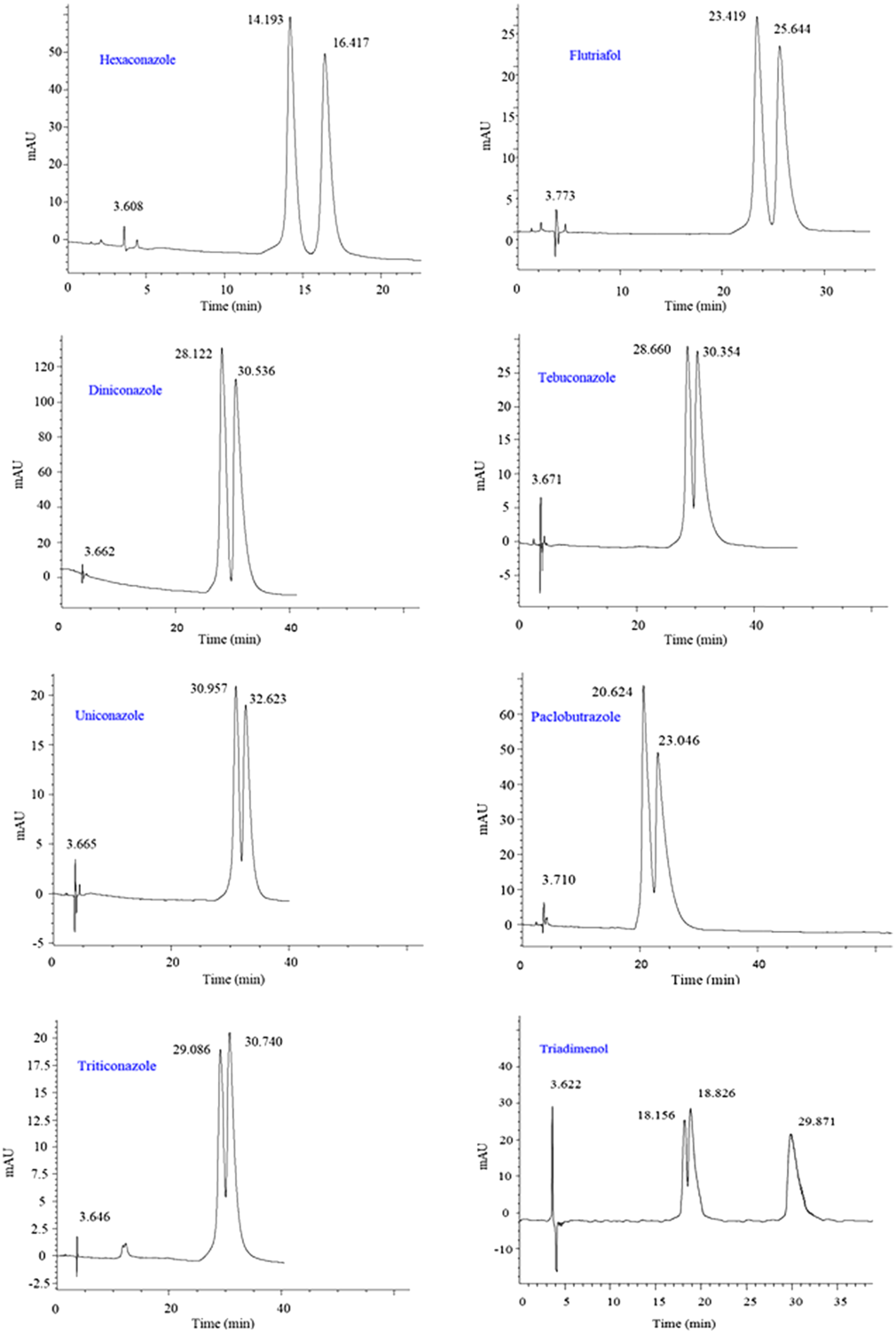

FIGURE 5 The chromatograms of some triazole pesticide enantiomers on triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase (TBCDP)

weakened the hydrogen bonding, but acetonitrile was aprotic solvent, so that the mixed organic modifiers methanol and acetonitrile could further the separation, but the ratio of methanol to acetonitrile required to be adjusted properly (Table 1).

Based on the chromatographic behavior of the above triazole pesticides and compared with CDSP, it can be found that TBCDP has a better chiral chromatographic performance for the selected triazole pesticides due to its synergistic inclusion ability.

3.2.2 | Chiral separation of flavanones

Chiral flavanones have extensively existed in medicinal herbs and possessed various therapeutic effects on inflammation, tumor, HIV virus, cardiovascular diseases, etc. It is of great significance to resolve natural chiral active ingredients. We here applied TBCDP to separate eight flavanones (Figure 6). Because this kind of analytes contain acidic phenolic hydroxyl groups, we found that the mobile phase containing 0.1% FA was the better eluent for their chiral resolutions. By changing the content of methanol or acetonitrile to optimize the resolutions of flavanones, it can be observed that chiral separation can be achieved in both organic modifiers, but acetonitrile was better than methanol, which is related to the structures of different flavanones. Table 2 listed the separation results and optimized chromatographic conditions, as well as some representative chromatograms were shown in Figure 7.

As shown in Table 2, the TBCDP had high enantioresolution (*R*s, 1.35-5.40) for all of the selected eight flavanones by using simple mobile phase in RP mode. Among them, the resolutions of 2′-hydroxyflavanone, 4′-hydroxyflavanone, catechin, and naringin were up to 5.40, 2.37, 2.60, and 3.02, respectively. However, CDSP only separated four flavanones with lower resolutions (1.12-1.69) than TBCDP. Despite higher bonding amount of CDSP (0.28 μmol/m^2) than TBCDP (0.14 μmol/m^2), its chiral separation ability was poor. In addition, because both of the two CD-CSPs were not derivatized, the chiral separation driving force should be the inclusion effects of the cavity and the hydrogen bonding of the ports of cyclodextrins.[17,18] Such difference in separation performance of TBCDP and CDSP should be related to different inclusion modes.[27] TBCDP could form an organic whole with clip-like shape through its two cavities and a flexible bridging group.[17] When the guest interacted with the bridged cyclodextrins, the entire the guest was enclosed by the two cavities and a bridging group of TBCDP, which facilitated the finer identification of the guest molecules as a whole, including the recognition of the spatial structure differences between the *R*- and *S*-enantiomers.[28] However, the cavity of the native β-CD was limited in size (0.65 nm) at most capable of encasing a naphthalene ring, while the bridged cyclodextrins was free from this confine and could form a synergistic inclusion complex with a larger volume of the guest, thus possessing stronger chiral recognition than the single cyclodextrins.

As can be seen from Table 2 and Figure 7, TBCDP could resolve all of the selected flavanones, including the parent flavanone (*R*s = 1.37), but the resolutions were significantly improved when the parent ring was replaced by the more hydroxyl groups. By comparing 2′-hydroxyflavanone (*R*s = 5.40), 4′-hydroxyflavanone (*R*s = 2.37), catechin (*R*s = 2.60), and naringin (*R*s = 3.02), it could be obviously found that the hydroxyl groups on the aromatic ring participated in chiral recognition. When this kind of analytes was encapsulated by the TBCDP, the hydrogen bonding would form between the hydroxyl groups of analytes and of the cyclodextrins' ports, which could help to make bigger difference in stability between *R*- or *S*-flavanone complexes and cyclodextrins to achieve better enantioseparation. However, CDSP was quite different in this case because it relied completely on a single and small cavity (0.65 nm), which

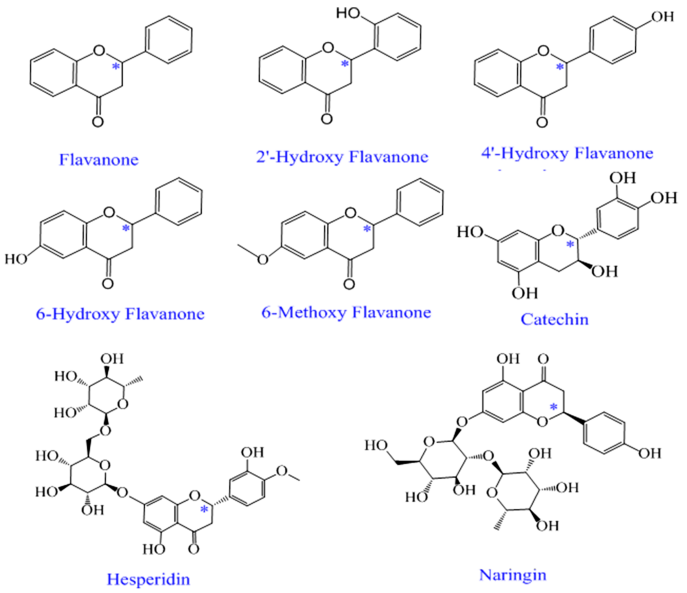

FIGURE 6 The chemical structures of eight flavanones

TABLE 2 The enantioseparation results of flavanones and better chromatographic conditions by HPLC

Compounds	k'_1	k'_2	α	*R*s	Mobile Phases	Columns
Flavanone	6.46	6.97	1.08	1.37	0.1% HCOOH/ACN (85/15, *v/v*)	TBCDP
	2.83	2.98	1.05	/	0.1% HCOOH/ACN (80/20, *v/v*)	CDSP
2′-Hydroxy flavanone	3.88	5.40	1.39	5.40	0.1% HCOOH/MeOH (65/35, *v/v*)	TBCDP
	2.54	2.98	1.17	1.69	0.1% HCOOH/MeOH (67/33, *v/v*)	CDSP
4′-Hydroxy flavanone	5.44	6.30	1.16	2.37	0.1% HCOOH/ACN (85/15, *v/v*)	TBCDP
	3.87	4.54	1.17	1.39	0.1% HCOOH/ACN (80/20, *v/v*)	CDSP
6-Hydroxy flavanone	6.15	6.47	1.05	1.43	0.1% HCOOH/MeOH (70/30, *v/v*)	TBCDP
	2.89	3.03	1.05	/	0.1% HCOOH/MeOH (65/35, *v/v*)	CDSP
6-Methoxy flavanone	8.75	9.38	1.07	1.38	0.1% HCOOH/MeOH (70/30, *v/v*)	TBCDP
	2.57	2.70	1.05	/	0.1% HCOOH/MeOH (65/35, *v/v*)	CDSP
Catechin	5.27	6.07	1.15	2.60	0.1% HCOOH/ACN (87/13, *v/v*)	TBCDP
	4.46	5.32	1.19	1.36	0.1% HCOOH/ACN (85/15, *v/v*)	CDSP
Hesperidin	6.01	6.54	1.09	1.35	0.1% HCOOH/ACN (93/7, *v/v*)	TBCDP
	4.93	5.08	1.03	/	0.1% HCOOH/MeOH (80/20, *v/v*)	CDSP
Naringin	3.54	4.32	1.22	3.02	0.1% HCOOH/ACN (93/7, *v/v*)	TBCDP
	2.54	3.12	1.23	1.12	0.1% HCOOH/MeOH (80/20, *v/v*)	CDSP

Abbreviations: CDSP, cyclodextrin stationary phase; HPLC, high-performance liquid chromatography; TBCDP, triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase.

made it difficult in the encapsulating of larger-volume analytes. For example, CDSP could not resolve 6-hydroxyflavanone, 6-methoxyflavanone, and hesperidin. Conversely, the resolutions (*R*s, 1.43, 1.38, and 1.35) for 6-hydroxyflavanone, 6-methoxyflavanone, and hesperidin on TBCDP were achieved, respectively (Table 2). Therefore, the bridged β-CDs has advantages in improving chiral recognition by synergistic inclusion.

3.2.3 | Chiral separation of dansyl amino acids

The amino acids, containing an acidic carboxyl groups and basic amino groups, are a kind of typical amphoteric chiral compounds not only serving as the basic blocks to constitute proteins but also serving as the intermediates of many important synthetic chiral drugs. Therefore, developing the new methods for the resolution and determination of amino acid enantiomers are of great significance. For the purpose of being easily resolved and detected, the amino acid is usually dansylated.[18] In this study, eight common *DL*-dansylated amino acids were used as probes to further investigate the chromatographic properties of TBCDP for amphoteric chiral compounds. The chemical structures of the selected dansyl amino acids were shown in Figure 8. The separation results and some chromatograms were shown in Table 3 and Figure 9.

It was usually necessary to use the buffer solutions in the mobile phase, which allowed the dansyl amino acids the proper ionization states for separations. The triethylammonium acetate (TEAA) is the most common buffers in the separations of dansyl amino acids. Wang et al[30] successfully resolved some of dansyl amino acids on the derivatized CD-CSPs by using TEAA-containing mobile phase with appropriate pH values. They believed that the native CD-CSPs could provide only insufficient separations for dansyl amino acids, and an effective resolution mainly depended upon the synergistic interactions of the derivatization groups and cavities.

According to the above reports, 1% TEAA-MeOH was selected as mobile phase to separate dansyl amino acids on TBCDP. The chromatographic conditions were optimized through the further investigation of some factors such as the concentration of organic solvents and pH values in mobile phases shown in Figure S3. Taking the acidic Dns-Asp as an example, it could be found that both the methanol content and pH values in mobile phase had optimal ranges (25% (*v/v*) MeOH, pH = 5.0) in terms of the Dns-Asp. Other amino acids were optimized by similar methods. The results are shown in Table 3.

As shown in Table 3, TBCDP could separate all of the selected eight dansyl amino acids (*R*s, 1.07-3.25) within 20 minutes (Figure 9), whereas the CDSP only separated six dansyl amino acids and with the lower resolutions (*R*s, 0.65-0.88). Obviously, TBCDP was more efficient in separating these analytes than CDSP. For examples, when used the TBCDP, the resolutions of Dns-Leu, Dns-Phe and Dns-Tyr respectively arrived at the 2.66, 2.48, and 3.25. These three Dns-amino acids were typical hydrophobic amino acids with fatty chains or benzene

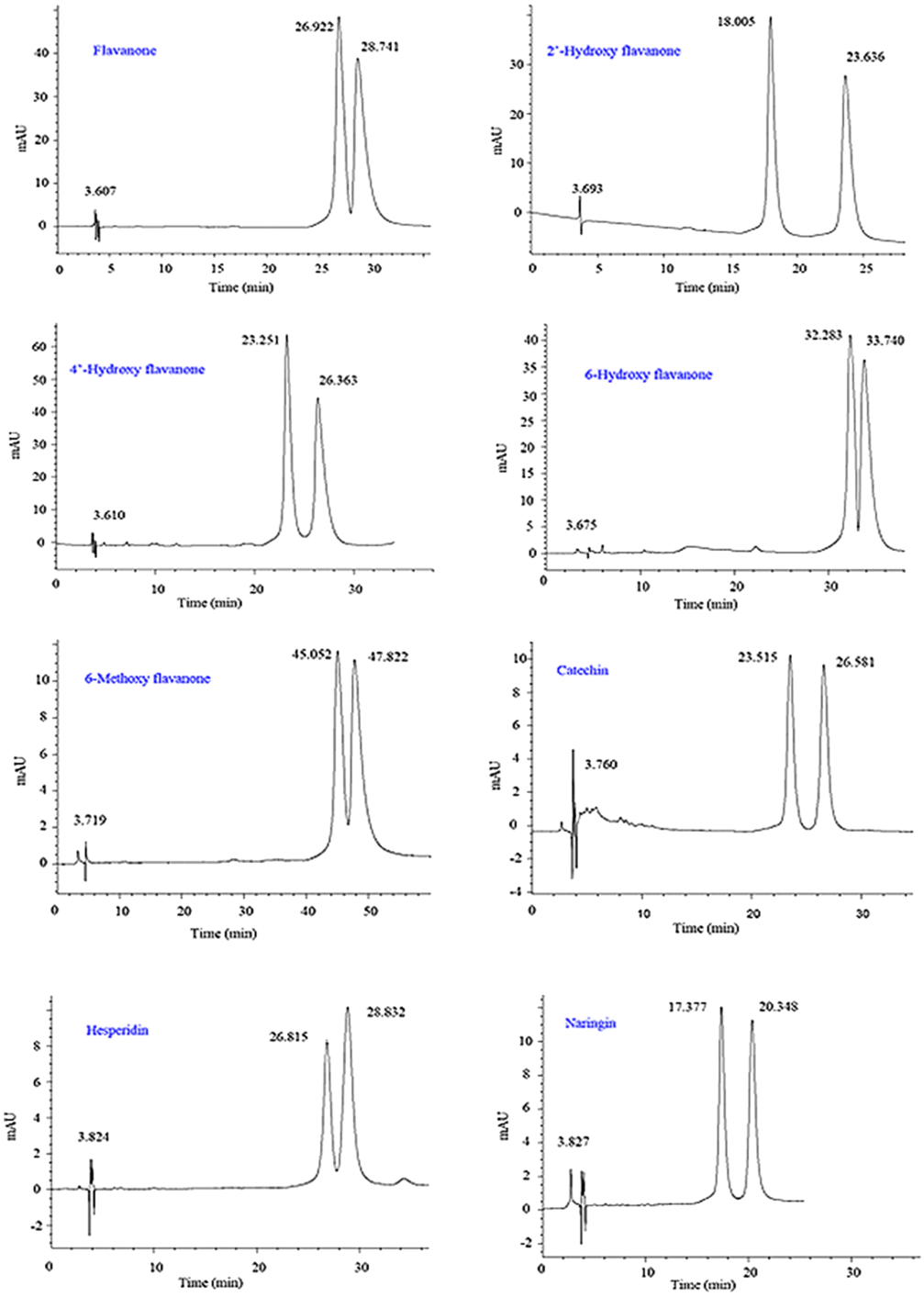

FIGURE 7 The chromatograms of some flavanone enantiomers on triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase (TBCDP)

rings that tend to be encapsulated by hydrophobic cavities of the cyclodextrin, thus facilitating chiral separations. This was consistent with the reported results of Wang research group,[32,33] which indicated that the separation of dansyl amino acids should mainly depend on the inclusion effects. In addition, encouraging results was that the TBCDP exhibited higher enantioresolutions for hydrophilic amino acids such as Dns-Ser, Dns-Thr, and Dns-Asp, containing polar hydroxymethyl, hydroxyethyl, and carboxymethyl groups, respectively. In general, these hydrophilic analytes are not easy to be encapsulated by hydrophobic cavities of the cyclodextrin; it is common to see that the derivatized CD-CSPs has incapable to separate these compounds.[30] However, the above Dns-amino acids were separated at the level of baseline resolutions by TBCDP (Figure 9), which further confirmed the advantage of the bridged cyclodextrins in chiral separation.

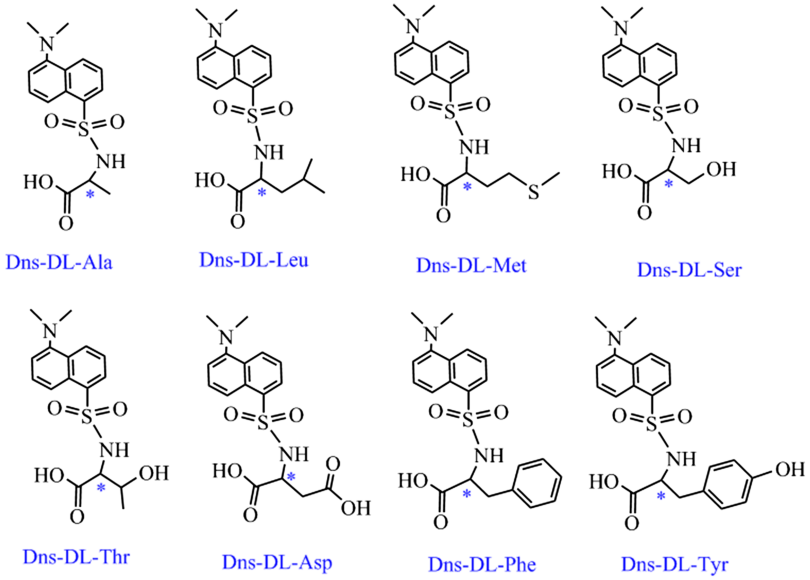

FIGURE 8 The chemical structures of eight dansyl amino acids

TABLE 3 The enantioseparation results of dansyl amino acids and better chromatographic conditions by HPLC

Compounds	k'_1	k'_2	α	Rs	Mobile Phases	CSPs
Dns-DL-Ala	1.64	1.75	1.07	1.07	1% TEAA/MeOH, 65/35, pH 5.0	TBCDP
	2.19	2.21	1.01	0.65		CDSP
Dns-DL-Leu	2.00	2.59	1.30	2.66	1% TEAA/MeOH, 60/40, pH 5.0	TBCDP
	1.16	1.21	1.04	0.97		CDSP
Dns-DL-Met	2.03	2.22	1.09	1.28	1% TEAA/MeOH, 65/35, pH 5.0	TBCDP
	1.49	1.58	1.06	0.70		CDSP
Dns-DL-Ser	1.41	1.57	1.11	1.52	1% TEAA/MeOH, 65/35, pH 5.0	TBCDP
	1.47	/	/	/		CDSP
Dns-DL-Thr	2.74	3.17	1.16	2.11	1% TEAA/MeOH, 75/25, pH 5.0	TBCDP
	1.37	/	/	/		CDSP
Dns-DL-Asp	3.55	3.88	1.09	1.45	1% TEAA/MeOH, 75/25, pH 5.0	TBCDP
	1.24	1.34	1.08	0.82		CDSP
Dns-DL-Phe	2.74	3.40	1.24	2.48	1% TEAA/MeOH, 60/40, pH 5.0	TBCDP
	1.75	1.82	1.04	0.87		CDSP
Dns-DL-Tyr	3.34	4.61	1.38	3.25	1% TEAA/MeOH, 70/30, pH 5.0	TBCDP
	1.45	1.62	1.11	0.88		CDSP

Abbreviations: CDSP, cyclodextrin stationary phase; CSPs, chiral stationary phases; HPLC, high-performance liquid chromatography; TBCDP, triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase.

3.2.4 | Chiral separation of β-blockers

The β-blocker is a class of chiral drugs that has been widely used in the treatment of cardiovascular diseases. Due to the rapid development of the species, there is no systematic enantiomeric monitoring method. Therefore, it is necessary to develop new materials to separate and determine the enantiomers of β-blockers. We herewith used TBCDP to separate ten chiral β-blockers (Figure 10). Initially, the chiral separation performed in RP mode, but it was found that no chiral separation occurred in this case, which mainly caused by the water of mobile phase that would destroy the hydrogen bonds. After switching to the RP mode, the selected β-blockers were successfully resolved on TBCDP. The results and the optimized separation conditions were listed in Table 4, as well as some chiral chromatograms in Figure 11.

Armstrong et al[14] firstly discovered the PO mode with using ACN/MeOH/HOAc/TEA as the mobile phase to separate anticholinergic, non-steroidal anti-inflammatory drugs and other chiral drugs that were difficult to be resolved under the aqueous RP mode. In this experiment, the effects of the ratio of ACN/MeOH and HOAc/TEA on the enantiomeric separations of these β-blockers were investigated (Figure S4), the results indicated that the methanol could accelerate the elution rate, but excess methanol could reduce the resolution significantly. In addition, adding a small amount of TEA could reduce the solute retention and make the shapes of enantioseparation peaks of these selected alkaline β-blockers more symmetrical; as well as an appropriate amount of HOAc could improve resolution. Therefore, the content of HOAc/TEA should be adjusted in an appropriate ratio. After optimizing chromatographic conditions, the good enantioseparations of the ten selected blockers on TBCDP were successfully achieved in the PO mode (*Rs*, 0.50-1.71) (Table 4), while the CDSP only separated six β-blockers with lower resolutions (*Rs*, 0.55-0.96).

The chiral carbon of β-blockers possesses the aminopropanol structure (–N–CH_2–C*HOH–CH_2–). The

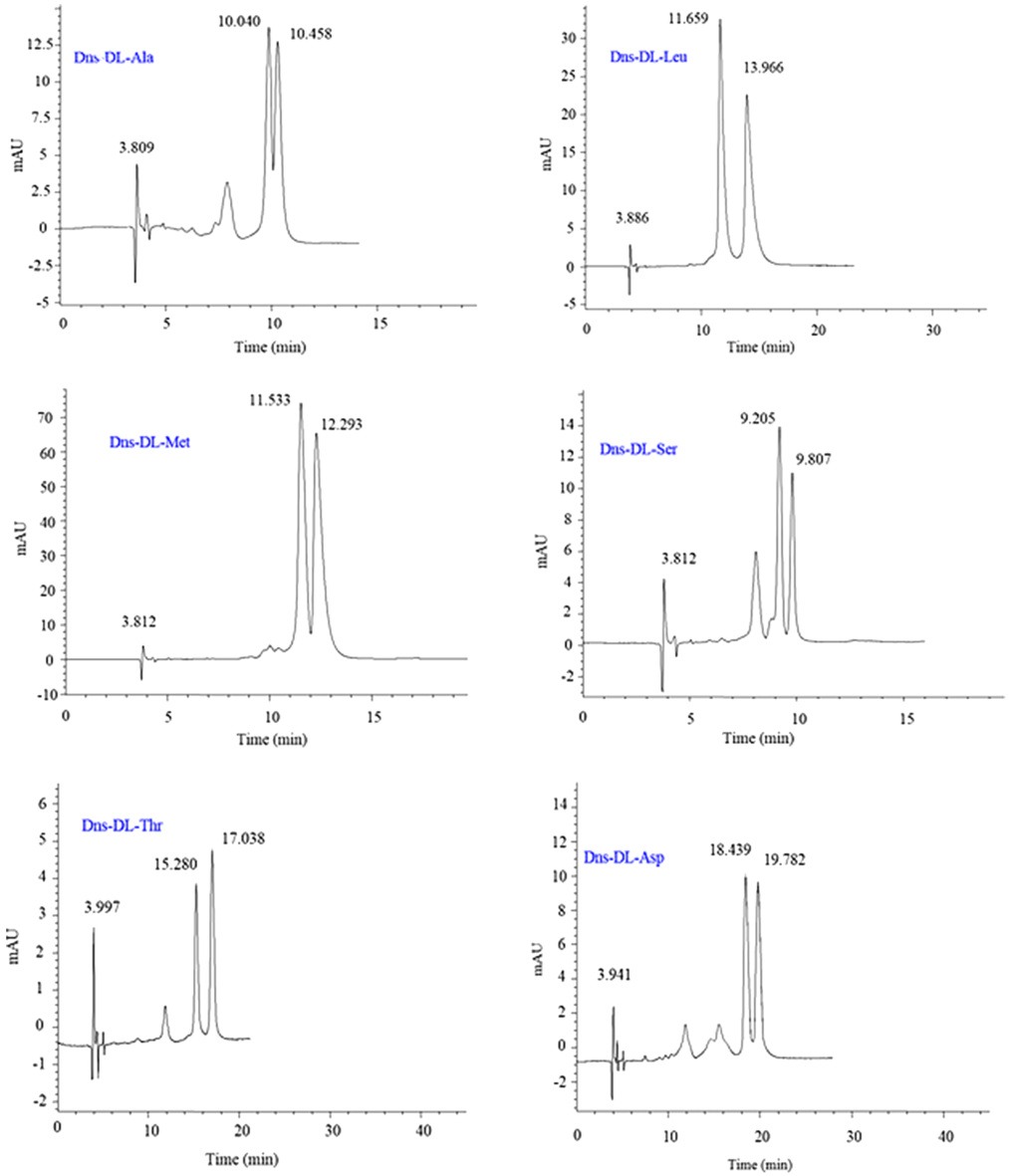

FIGURE 9 The chromatograms of some dansyl amino acid enantiomers on triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase (TBCDP)

–OH on their chiral carbons could form hydrogen bonding with –OH on the cyclodextrins, which was the key effects in the chiral separation. Since water was a powerful hydrogen bond donor that would greatly weaken the hydrogen bonding, the RP chromatographic mode had incapability to separate β-blockers.[18,20] The PO mode with free from water was favorable for hydrogen bonding for chiral separation of β-blockers. For example, both atenolol (*Rs* = 1.46) and arotinolol (*Rs* = 1.71) contained amide groups that had stronger hydrogen bonding with TBCDP, thus prolonging the retention times (elution of methanol required about 10%), as well as increasing the resolutions.

In addition to hydrogen bonding, inclusion also contributed to the above resolutions significantly.[26,32,33] From the resolutions of atenolol (*Rs* = 1.46), arotinolol (*Rs* = 1.71), metoprolol (*Rs* = 1.56), and esmolol (*Rs* = 1.47) (Figure 10), we concluded that these linear structures would easily to enter the cavities of TBCDP. Although the similarity in structure of bisoprolol and metoprolol, there was a significant gap in resolutions of bisoprolol (*Rs* < 0.5) and metoprolol (*Rs* = 1.56). The reason may be due to the substituent chain on the benzene ring of bisoprolol was too long to be encapsulated by the cyclodextrins, because its enantiomer-resolution significantly decreased (<0.5). Likewise, except for aromatic ring, propranolol was very similar to carteolol in the structure (Figure 10), but the resolution (*Rs* = 1.33) of propranolol was significantly higher than that of carteolol (*Rs* = 0.57). This may be attributed to the fact that

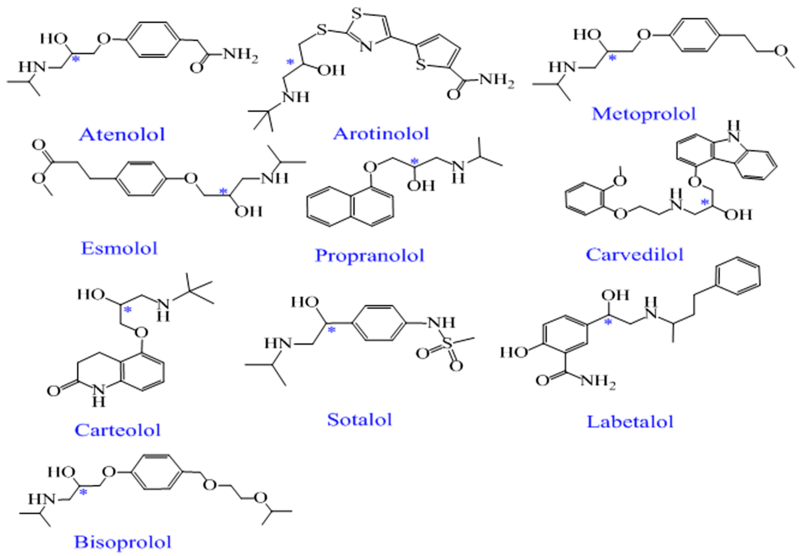

FIGURE 10 The chemical structures of ten chiral β-blocker drugs

TABLE 4 The enantioseparation results of β-blockers and better chromatographic conditions by HPLC

Compounds	k'_1	k'_2	α	*Rs*	Mobile Phases	CSPs
Atenolol	7.88 2.67	8.96 4.99	1.14 1.07	1.46 0.55	ACN/MeOH/HAc/TEA (90/10/1/0.9)	TBCDP CDSP
Arotinolol	7.64 4.67	9.27 5.18	1.21 1.11	1.71 0.82	ACN/MeOH/HAc/TEA (93/7/1/1)	TBCDP CDSP
Metoprolol	3.00 1.54	3.42 1.67	1.14 1.08	1.56 0.68	ACN/MeOH/HAc/TEA (95/5/1/0.4)	TBCDP CDSP
Esmolol	2.63 2.31	2.98 2.41	1.13 1.04	1.47 0.65	ACN/MeOH/HAc/TEA (95/5/0.4/0.4)	TBCDP CDSP
Propranolol	3.90 2.95	4.35 3.15	1.12 1.06	1.33 0.96	ACN/MeOH/HAc/TEA (94/6/1/1)	TBCDP CDSP
Carvedilol	4.80 3.75	5.49 /	1.14 /	1.30 /	ACN/MeOH/HAc/TEA (99.4/0.6/1/1)	TBCDP CDSP
Carteolol	3.93 2.88	4.14 /	1.05 /	0.57 /	ACN/MeOH/HAc/TEA (97/3/0.5/0.6)	TBCDP CDSP
Sotalol	5.06 3.44	5.23 /	1.03 /	<0.5 /	ACN/MeOH/HAc/TEA (95/5/0.9/0.8)	TBCDP CDSP
Labetalol	3.75 2.89	3.89 /	1.04 /	0.54 /	ACN/MeOH/HAc/TEA (94/6/0.9/1)	TBCDP CDSP
Bisoprolol	2.45 1.94	2.56 /	1.04 /	<0.5 /	ACN/MeOH/HAc/TEA (96/4/1/1)	TBCDP CDSP

Abbreviations: CDSP, cyclodextrin stationary phase; HPLC, high-performance liquid chromatography; TBCDP, triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase.

hydrophobic naphthalene ring of propranolol could be easily entrapped by the cavity of TBCDP, while the polar benzocaprolactam ring of carteolol could not match with the hydrophobic cavity that caused the inclusion effect of TBCDP weakened and decreased the resolution of carteolol. In general, TBCDP had potential enantioseparation ability for some bulky and more complex analytes such as carvedilol and labetalol, which indicated that the synergistic inclusion effect and other potential effects of the bridged CD-CSPs could identify such chiral β-blocker drugs.

Based on the above chromatographic evaluation data, it could be concluded that the bridged CDSP (TBCDP) had a stronger chiral separation ability and wider separation range than the single CDSP. When analytes interacted with the bridged cyclodextrin, the entire guest could be

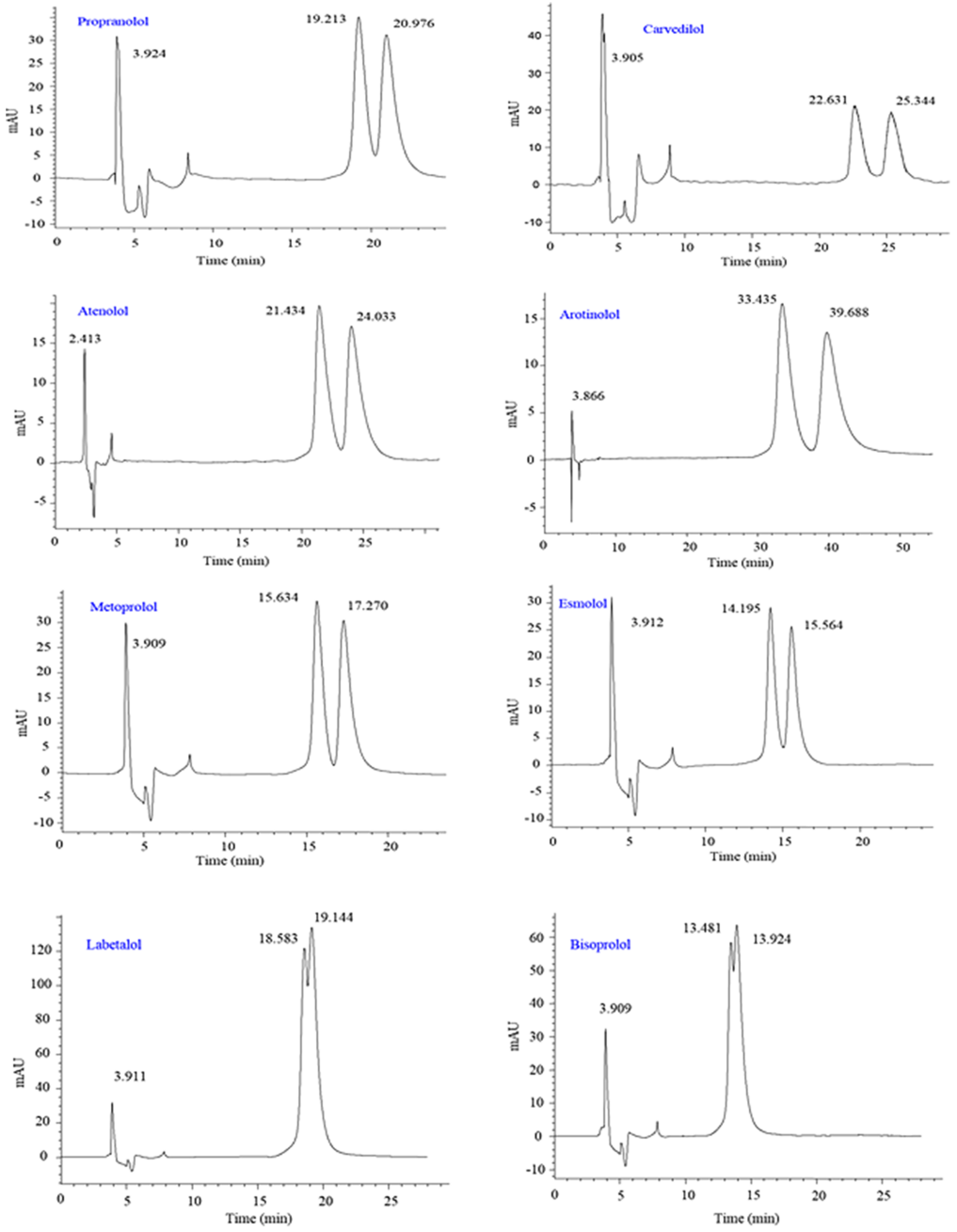

FIGURE 11 The chromatograms of some β-blocker drug enantiomers on triazole-bridged *bis*(β-cyclodextrin)–bonded chiral stationary phase (TBCDP)

cooperatively encapsulated by the two cavities and a linker group of the bridged cyclodextrin host. This facilitated the finer identification of the guest molecules as a whole, including the recognition of the spatial structure differences between the *R*- and *S*-enantiomers.

4 | CONCLUSION

In this paper, a novel TBCDP was prepared by a *Click Chemistry* reaction. The preparation method was simple, rapid, and efficient, and the as-prepared TBCDP had a high chemical stability and a good chromatographic reproducibility. The common chiral triazole pesticides and chiral drugs including flavanones, β-blockers, and dansyl amino acids had been successfully resolved under the RP and PO modes with encouraging results. By constructing a new supramolecular host with the two cavities and a bridging linker without derivatization, the synergistic inclusion, hydrogen bond, steric hindrance, and π-π were introduced to chiral separations, thus improving the enantioselectivity of TBCDP. Therefore, the bridged CDSP was a kind of multimode stationary phase with potentially application value.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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