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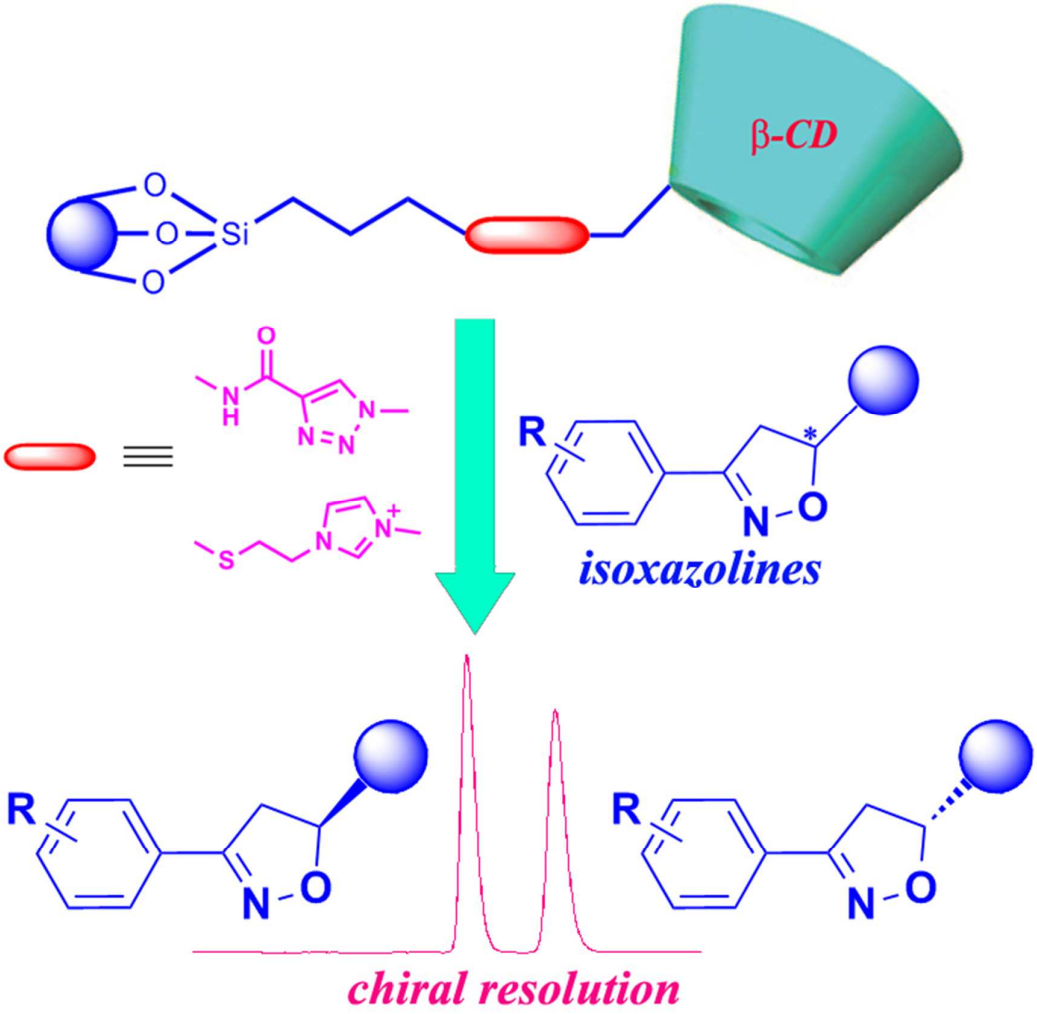

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PAPER

Chiral differentiation of novel isoxazoline derivatives on "clicked" thioether and triazole bridged cyclodextrin chiral stationary phases

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Isoxazoline derivatives have been disclosed in the art as having acaricidal and insecticidal activity, and as potential precursors for the syntheses of natural products. This work firstly demonstrates the chiral resolution of isoxazoline derivatives that had not been studied before on native cyclodextrin (CD) chiral stationary phases (CSPs). Two structurally-well defined CSPs based on native CD were prepared via different click procedures and applied for enantioseparation of isoxazolines. Most of the studied isoxazolines were found to be well resolved (R_s > 1.5) under reversed phase mode, especially for 4NPh-OPr which exhibits the best enantioselectivity and resolution (α = 2.22; R_s = 4.16). Optimal resolutions were achieved by evaluating the influences of mobile phase composition, substitution moieties and CSP linkages on the separation. This contribution verifies that excellent enantioseparation of isoxazolines can be accomplished on smartly designed native CD-CSP, which provides a facile and economic way to obtain enantiopure isoxazoline derivatives.

1. Introduction

Compounds incorporating heterocyclic rings continue to attract considerable interest owing to the wide range of their interesting biological activities. Amongst them, five-membered heterocyclic compounds occupy a conspicuous place in the field of natural and synthetic organic chemistry. Isoxazolines, as a class of five-membered heterocyclic compounds, have found wide applications as agrochemical agents and pharmaceuticals. Such compounds possess various biological activities such as insecticidal, antibacterial, antibiotic, antitumour, antifungal, antimicrobial, vitro anti-tuberculosis activity and anti-inflammatory activities.[1-9] In addition, isoxazoline derivatives have been recognized as important intermediates for the synthesis of alkaloids and other natural products.[10, 11]

Small pharmaceutical compounds with substituted isoxazoline rings such as some phenylisoxazolines exhibit high activity against gram-positive pathogens.[12] Series of 3-substituted-5-pyridinyl-isoxazolines can be used as protein tyrosine phosphatase 1B inhibitors.[13, 14] Researchers keep dedicating great efforts to the development of novel isoxazoline derivatives. More and more products based on isoxazole nucleus have been designed and synthesized. Our previous study described the synthesis of a variety of novel isoxazolines via 1,3-dipolar cycloaddition reaction between mono-substituted alkenes and nitrile oxides.[15]

As it is known, the bioactivity of chiral compounds is closely related with its stereochemistry. Isoxazoline enantiomers in agrochemicals can have diverse effects on plants and insects, and could bring out negative effects to the environment and be toxic to human health. Hence, it's necessary to get optically pure isoxazolines to make best use of their performance and minimize potential harms.[16-19] Since the catalytic asymmetric synthesis is tedious and difficult, most isoxazolines are generated in lab with equal amounts of enantiomers in achiral environments. As a result, it is highly desirable to build efficient and cost effective enantioseparation approaches to get the enantiopure isomers from the abundant isoxazoline racemates.

On a separate note, cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides consist of several (6, 7, 8) glucose units, which are one of the most commonly used economic chiral selectors for enantioseparation due to their ability to form 'host-guest' inclusion with a large variety of chiral compounds. Since Fujimura successfully synthesized CD-CSPs based on amino linkages for the first time in 1983, it has caught great interests in separation area. Chemists are constantly searching for new synthetic methods for the preparation of stable CD-CSPs. Numbers of CD-CSPs with various linkages have been developed, such as ether linkages by Amstrong,[20] urea linkages by Ng[21, 22] and triazole linkages by Liang and Ng.[23-27]

In recent years, "click chemistry" is attracting more and more interest owing to its advantages such as insensitivity to water and oxygen as well as the mild reaction conditions. Typical representations are Cu(I) catalytic 1,3-dipolar cycloaddition

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reaction and radical-based thiol-ene reaction, which have been widely used in the preparation of various stationary phases.[28-30] Great importance was addressed to triazole linkaged CD-CSPs that prepared by 1,3-dipolar cycloaddition reaction owing to its simple synthesis, controllable structure, good stability and powerful resolving ability. Our group has employed thiol-ene click reaction constructing a novel cationic native CD-CSP for enhanced separation of acidic chiral compounds in HPLC.[31] Both the two click reactions provide facile and economic approaches to obtain structurally well-defined CD-CSPs.

In this work, we prepared two native CD-CSPs with triazole and thioether linkages via the above described click procedures and performed enantioseparation of twenty-six novel isoxazoline enantiomer pairs such as 3-aryl-5-phenyl-isoxazolines (Ar-Ph), 3-aryl-5-(pyridin-4-yl)-isoxazolines (Ar-Py) and 3-aryl-5-(2-oxopyrrolidin-1-yl)-isoxazolines (Ar-POr) by reversed phase high performance liquid chromatography (HPLC). To the best of our knowledge, this is the first report on chiral resolution of isoxazoline enantiomers using native CD-CSPs, which is expected to provide a facile and economic way to obtain enantiopure isoxazoline derivatives at both analytical and preparative levels.

2. Experimental

2.1 Chemicals and materials

For CSP preparation: azobisisobutyronitrile (AIBN) was purchased from Tianjin Chemical Reagents (Tianjin, China), 3-mercaptopropyltrimethoxysilane and 1-allylimidazole were purchased from Energy-Chemical (Shanghai, China), Mono-6^{A}-deoxy-(*p*-tolylsulfonyl)-β-cyclodextrin (TsO-CD) was synthesized according to the reported procedure.[32] Anhydrous *N,N*-dimethylformamide (DMF) and toluene were provided by Heowns (Tianjin, China). Kromasil spherical silica gel (5 μm, 100 Å) was obtained from Eka Chemicals (Bohus, Sweden).

For chromatographic experiments: HPLC-grade methanol (MeOH), acetonitrile (ACN), triethylamine (TEA) and acetic acid were provided by Guangfu chemical reagents (Tianjin, China). Ultra-pure water was prepared by Milli-Q water purification system (Billerica, MA, USA). All the isoxazoline racemic pairs used were synthesized according to our previous reported procedure[15] and their structures are shown in Fig.1.

2.2 Preparation of CSPs

Triazole bridged CD-CSP (**CSP1**) and thiolether bridged CD-CSP (**CSP2**) (Fig.2) were synthesized according to the methods described in the previous reports.[31, 32] The detailed synthetic procedures were included in the supporting information (SI). The linkage of **CSP1** can provide hydrogen bonding sites via oxygen and nitrogen as well as dipole-dipole interactions; the linkage of **CSP2** affords electrostatic force on account of cationic heterocyclic ring. The surface coverage of CD was calculated as 0.85 μmol·m^{-2} and 0.60 μmol·m^{-2}, respectively according to the following equation:

$$\frac{\mu mol}{m^2} = \frac{(\%C)(10^6)}{(S.A)(n_c)(12.001)}[100 - \frac{\%C}{(n_c)(12.001)}(M_r)]$$

S.A is the surface area of the silica; n_c is the carbon numbers; M_r is the molecular weight, *C%* is from elemental analysis results (SI).

The particle size distribution of the prepared CSP is displayed in Fig.S3 (SI). The electron microscopy images of bare silica and CSP are illustrated in Fig.S4 (SI). Most of the particle size falls on the range from 3 to 7 μm and the morphology of silica particles does not change a lot before and after reaction.

2.3 Column packing

The prepared CSPs were dispersed in MeOH followed by packing into stainless-steel column (150 mm × 4.6 mm I.D.) using typical slurry-packing technique with MeOH as the packing solvent. The packing session lasted for 30 minutes at constant pressure of 5000 psi. The column efficiency for the studied analytes calculated by USP standard.

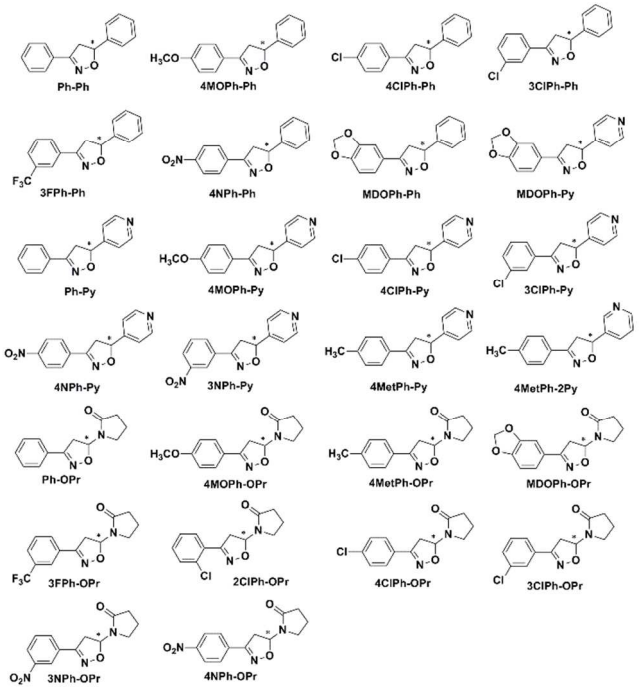

Fig.1 Structures of isoxazoline derivatives

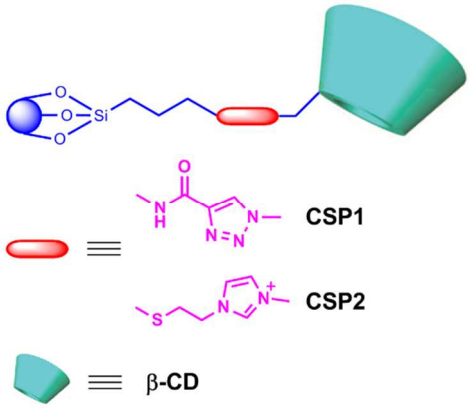

Fig.2 Structures of triazole and thioether bridged CD-CSPs

2.4 Instruments and chromatographic conditions

Chromatographic analyses were performed on a Laballiance HPLC system with a diode array detection (DAD) system (State college, PA, USA). ACN/MeOH and deionized water were used as mobile phases (MP). Samples were dissolved in MeOH/H_2O (v/v = 1:1) at a concentration of 1 mg·mL^{-1} and the injection

volume was set as 1 μL. All the MP and samples were filtered through a 0.22 μm membrane before usage. The detection was performed at 220–300 nm. Each solution was injected in triplicate and the average value was used. Calculations for capacity factor k, selectivity α and resolution R_s were performed following USP standards: k was calculated using $k = (t_R - t_0)/t_0$, where t_R is the retention time of the enantiomers and t_0 is the dead time determined by measuring the first base-line perturbation. α was calculated using $\alpha = k_2/k_1$. R_s was calculated using the equation: $1.18\times(t_2 - t_1)/(W_{h1} + W_{h2})$ where W_h is the half peak width.

3. Results and discussion

3.1 Enantioseparation of isoxazoline derivatives on triazole-bridged CD-CSP (CSP1)

Initially, ACN/H_2O=40/60 (v/v) or MeOH/H_2O=50/50 (v/v) was chosen as the MP, and a part of the analytes can be baseline separated. By optimizing the separation conditions, most of the analytes were baseline separated within 40 min. The optimal separation results are listed in table 1 and several representative chromatograms are shown in Fig.3. More chromatograms are included in Fig.S5 (SI). The encouraging results demonstrate that **CSP1** afforded powerful resolving ability towards the studied isoxazoline derivatives.

Table 1. Optimal separation results of isoxazolines on **CSP1**

	Analytes	k_1	k_2	α	R_s	N_1	conditions
Ar-Ph	MDOPh-Ph	2.18	3.51	1.61	3.79	2163	
	Ph-Ph	1.76	2.68	1.52	3.05	2122	ACN/H_2O=40/60
	4ClPh-Ph	1.95	2.84	1.46	2.78	2092	
	4MOPh-Ph	5.77	8.17	1.42	3.80	3822	
	3ClPh-Ph	6.65	8.49	1.28	2.65	3668	ACN/H_2O=30/70
	4NPh-Ph	7.05	8.84	1.25	2.36	3642	
	3FPh-Ph	22.01	23.63	1.07	0.78	3479	ACN/H_2O=20/80
Ar-Py	4MOPh-Py	1.38	1.92	1.39	2.03	2594	ACN/H_2O=40/60
	4ClPh-Py	1.54	1.93	1.25	1.56	3450	
	MDOPh-Py	3.83	5.11	1.34	2.41	2618	
	4MetPh-Py	3.72	4.69	1.26	1.97	2706	ACN/H_2O=30/70
	Ph-Py	2.54	3.28	1.29	1.85	2348	
	3ClPh-Py	6.60	7.69	1.16	1.51	3056	ACN/H_2O=25/75
	4NPh-Py	11.16	12.74	1.14	1.46	3564	ACN/H_2O=20/80
	3NPh-Py	13.30	14.27	1.07	0.66	2720	ACN/H_2O=15/85
	4MetPh-2Py	2.56	3.37	1.32	2.29	3176	ACN/H_2O=30/70
Ar-OPr	4MetPh-OPr	1.44	1.87	1.29	1.60	2523	ACN/H_2O=30/70
	4ClPh-OPr	2.64	3.22	1.22	1.69	3266	
	4MOPh-OPr	1.85	2.33	1.26	1.63	2822	ACN/H_2O=25/75
	MDOPh-OPr	2.82	3.38	1.20	1.59	3341	
	Ph-OPr	3.43	4.15	1.21	1.87	3764	ACN/H_2O=20/80
	4NPh-OPr	3.75	4.43	1.18	1.63	3621	
	3ClPh-OPr	4.85	5.64	1.16	1.63	4008	
	3FPh-OPr	6.01	6.90	1.15	1.48	3707	
	2ClPh-OPr	9.62	10.75	1.12	1.32	4107	ACN/H_2O=15/85
	3NPh-OPr	3.79	4.14	1.09	0.79	3263	
	4NPh-OPr	2.41	5.36	2.22	4.16	2157	
	4MetPh-OPr	2.72	3.92	1.44	2.95	2478	
	4ClPh-OPr	2.62	3.88	1.48	2.87	2391	
	MDOPh-OPr	4.03	5.78	1.43	2.86	3027	MeOH/H_2O=50/50
	3ClPh-OPr	2.30	3.62	1.57	2.54	2246	
	4MOPh-OPr	2.22	3.11	1.40	2.37	2157	
	Ph-OPr	1.85	2.53	1.37	1.99	1967	
	3NPh-OPr	2.76	3.66	1.33	1.75	2159	MeOH/H_2O=40/60
	2ClPh-OPr	10.02	11.74	1.17	1.64	2952	MeOH/H_2O=30/70
	3FPh-OPr	8.37	9.63	1.15	1.40	3020	

Conditions: flow rate = 0.6 mL·min^{-1}, 25 °C

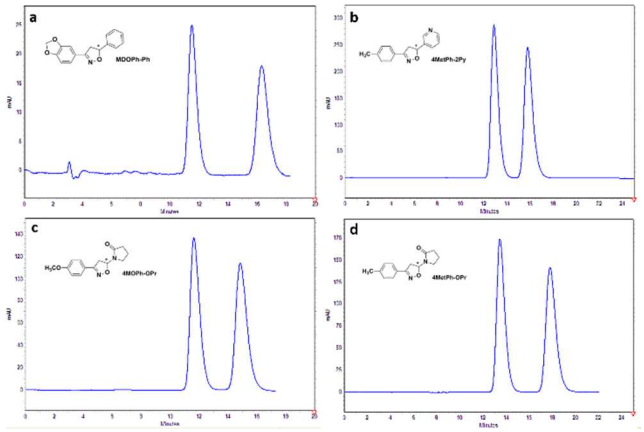

Fig.3 Representative chromatograms on **CSP1**. Conditions: ACN/H_2O=40/60 (a), ACN/H_2O=30/70 (b), MeOH/H_2O=50/50 (c, d); flow rate = 0.6 mL·min^{-1}; 25 °C

3.1.1 Effect of MP compositions on enantioseparation for CSP1

Since organic modifier plays key roles in enantioseparation on CD-CSPs, it is necessary to make clear which kind of organic modifiers is more favorable for the enantioseparation in this study. According to the substituents on 5-position of the isoxazoline ring, the analytes can be classified into three categories: Ar-Ph (phenyl ring on 5-position), Ar-Py (pyridine ring on 5-position) and Ar-OPr (pyrrolidinone ring on 5-position). To investigate the effect of organic modifier type, we chose several model analytes Ph-Ph, 4MOPh-Ph, MDOPh-Ph, Ph-Py, 4MOPh-Py, 4MetPh-Py and Ph-OPr, 4ClPh-OPr, 4ClPh-OPr (Fig.1) to conduct the enantioseparation experiments and the separation results are shown in table 2.

Table 2. Effect of MP compositions on **CSP1**

	analytes	conditions	k_1	k_2	α	R_s
Ar-Ph	Ph-Ph	I	1.76	2.68	1.52	3.05
		II	8.77	9.59	1.09	1.02
	4MOPh-Ph	I	0.90	1.09	1.20	0.73

		II	8.29	8.95	1.08	0.79
	MDOPh-Ph	I	2.18	3.51	1.61	3.79
		II	13.25	15.18	1.15	1.31
Ar-Py	Ph-Py	I	1.04	1.32	1.26	1.11
		II	3.55	4.01	1.13	1.00
	4MOPh-Py	I	1.38	1.92	1.39	2.03
		II	9.48	10.26	1.08	1.10
	4MetPh-Py	I	1.21	1.51	1.25	1.13
		II	4.61	5.46	1.18	1.40
Ar-OPr	Ph-OPr	I	0.43	0.52	1.21	0.28
		II	1.85	2.53	1.37	1.99
	4ClPh-OPr	I	0.46	0.57	1.23	0.41
		II	2.62	3.88	1.48	2.87
	4ClPh-OPr	I	0.44	——	≈—	≈—
		II	2.30	3.62	1.57	2.54

Conditions：flow rate = 0.6 mL/min, 25 ℃; (I) ACN/H_2O=40/60, (II) MeOH/H_2O=50/50.

As shown in table 2, Ar-Ph and Ar-Py are more strongly retained than Ar-OPr under same conditions due to the existence of two aryl moieties which can both form fit inclusion with the CD hydrophobic cavity. ACN and MeOH afford much different effects towards the separation of the three categories. For Ph-Ph and Ph-Py, especially Ph-Ph, although the retention is greatly weakened by transferring the MP from MeOH/H_2O to ACN/H_2O (k_1 from 8.31 to 2.24), the R_s experiences a dramatic increase from 1.02 to 3.05; while for Ph-OPr, methanol affords much better separation (R_s = 1.99) than ACN (R_s = 0.28). The reason could be attributed to the nature of ACN and MeOH. MeOH is a protic solvent which can form H-bonding with the analytes and CSP hence favoring the separation of the more polar Ph-OPr, while ACN is aprotic and better to resolve the more hydrophobic Ph-Ph and Ph-Py.

In addition, the effect of water content in the mobile phase was studied with some model analytes and the results were plotted in Fig.S6 (SI). With the decrease of water content in MP, the retention time firstly show a significant decline and so does the resolution, which is typical characteristic of the reversed-phase mode (RPLC). A slight increase of the retention is found at ACN content of 90%, indicating the separation mode starts to transit to hydrophilic chromatography (HILIC).

3.1.2 Enantioseparation of Ar-Ph category

Based on the results obtained above, ACN/H_2O was chosen as MP for separation of Ar-Ph category. The separation results under ACN/H_2O = 40/60 (v/v) are depicted in Fig.4.

When Ar-Ph interacts with CD-CSP, both the aromatic groups on 3- or 5-position can enter the CD cavity firstly to form inclusion complex, together with the additional hydrogen bonding, π–π interaction as well as steric effects, a three-point interaction model can be well established. As seen from Fig.4, MDOPh-Ph affords the strongest retention and best separation benefiting from the tight inclusion between the CD cavity and the enlarged hydrophobic part on 3-position. It is interesting to find that 4ClPh-Ph shows higher selectivity and resolution than 3ClPh-Ph, which indicates that *p*-position substitution by -Cl on phenyl ring affords better inclusion formation ability than *m*-position. The reduced retention and separation of 4NPh-Ph and 4MOPh-Ph suggests their loose inclusion with the CD cavity, which might be due to the steric hindrance effect. It was found that strong electron-withdrawing moieties like $–CF_3$ significantly diminish the separation ascribed to the increased polarity of the phenyl ring, which is reflected from the very poor resolution of 3FPh-Ph. Further optimization was conducted by reducing the ACN proportion to enhance the inclusion complexation. By gradually decreasing the ACN proportion from 50% to 30%, most of the analytes can be baseline resolved except 3FPh-Ph which was only partially separated even 20% ACN was used (table 1).

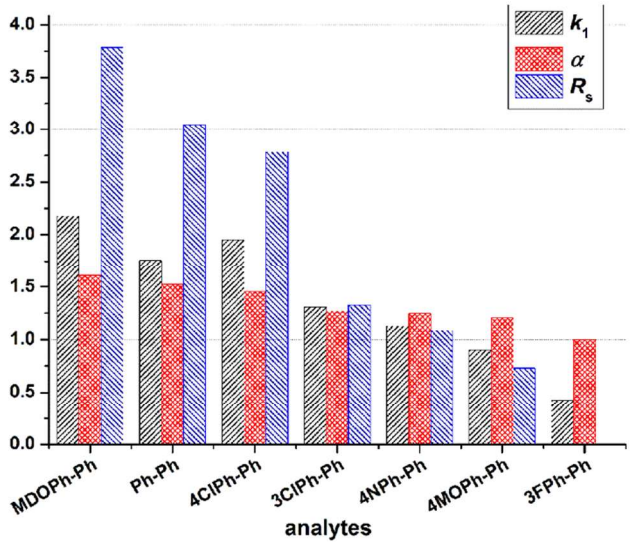

Fig.4 Enantioseparation of Ar-Ph on **CSP1**. Conditions: flow rate = 0.6 mL·min^{-1}, temperature = 25 °C

3.1.3 Enantioseparation of Ar-Py category

Similar to the separation of Ar-Ph category, substitute of -NO_2 on the phenyl ring strongly weakens the separation for Ar-Py category like 4NPh-Py and 3NPh-Py as shown in Fig.5 and 3,4-methylenedioxy group on MDOPh-Py is helpful to enhance the chiral differentiation. In addition, 4ClPh-Py also gains much better resolution than 3ClPh-Py. However, the –OMe on *p*-position strongly enhances the chiral recognition of Ar-Py (4MOPh-Py), which is different from Ar-Ph category. This suggests the slight difference in the inclusion formation mechanism between the two categories with CD cavity. The pyridine moiety of Ar-Py may form H-bonding with the –OH on CD rims, hindering its entry into the CD cavity to some extent. The different separation results of 4MetPh-Py and 4MetPh-2Py may evidence this speculation. Further optimization results have been listed in table 1. Most of the Ph-Py racemates can be completely resolved (R_s > 1.5) except 3NPh-Py.

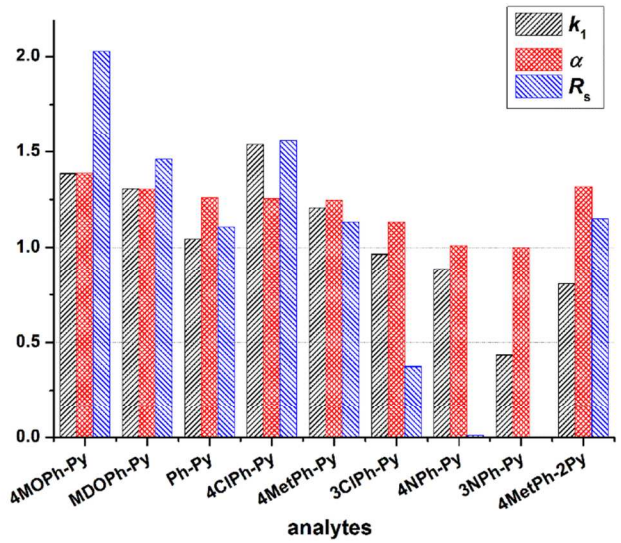

Fig.5 Enantioseparation of Ar-Py on **CSP1**. Conditions: flow rate = 0.6 mL·min^{-1}, temperature = 25 °C

3.1.4 Enantioseparation of Ar-OPr category

The enantioseparation of Ar-OPr category was performed with MeOH/H_2O as MP according to the results obtained previously. In order to illustrate the substituent effect of Ar-OPr, Fig.6 depicts the separation results of Ar-OPr with MP of MeOH/H_2O = 50/50 (v/v) and the optimized results have been included in table 1.

As it is found in Fig.6, the strongest retention falls on MDOPh-OPr due to the tight inclusion similar to the separation of MDOPh-Ph and MDOPh-Py, while the α and R_s of MDOPh-OPr are moderate. This phenomenon indicates that stronger inclusion does not necessarily bring about better chiral separation. As the pyrrolidinone moiety affords H-bonding, dipole-dipole interaction sites and steric effects, the binding geometry would play important role in the chiral differentiation process. The *p*-NO_2 phenyl ring of 4NPh-OPr may form the most favoring binding geometry with the CSP hence shows the best enantioselectivity (α = 2.22) and resolution (R_s = 4.16) while the -NO_2 at *m*-position decreases the separation remarkably. By comparison of 4ClPh-OPr, 3ClPh-OPr, 2ClPh-OPr and Ph-OPr, the retention and resolution follows an order of 4ClPh > 3ClPh > Ph > 2ClPh, indicating that -Cl on *p*- and *m*-positions favorites the separation while -Cl on *o*-position attenuates the chiral differentiation.

Further study was performed on enantioseparations with ACN/H_2O as MP was conducted. It was found that all the Ar-OPr enantiomers are better separated with MeOH than ACN (Fig.S1). This indicates the different recognition mechanisms of Ar-OPr, Ar-Ph and Ar-Py on CD CSPs. Protic organic modifiers are more suitable for the enantioseparation of isoxazolines with pyrrolidinone moieties.

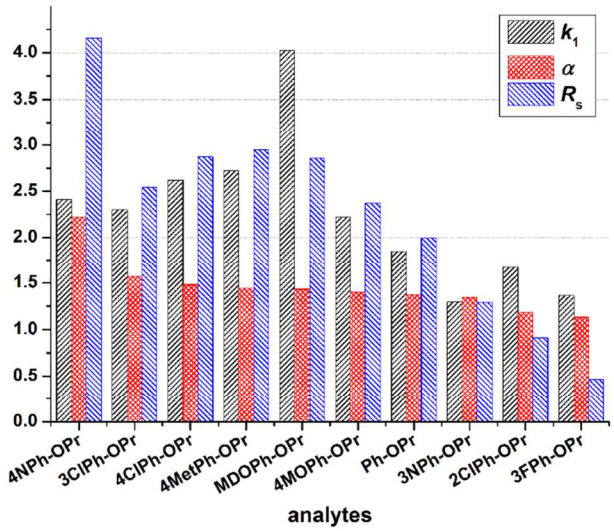

Fig.6 Enantioseparation of Ar-OPr on **CSP1**. Conditions: flow rate = 0.6 mL·min^{-1}, temperature = 25 °C

3.2 Enantioseparation of isoxazolines on thioether bridged CD-CSP (CSP2)

CSP2 presents similar recognition behaviors with **CSP1**. Ar-Ph and Ar-Py categories can be better separated in presence of ACN than MeOH, while the reversed situation was found with the separation of Ph-OPr category. By optimizing the separation conditions, most analytes of Ar-Ph category were baseline resolved (table 3) and typical chromatograms are depicted in Fig.7. As expected, MDOPh-Ph affords the best separation due to the fit inclusion. 4ClPh-Ph gains greater enantioselectivity than 3ClPh-Ph and 3FPh-Ph shows the poorest separation.

Table 3. Optimal separation results of isoxazolines on **CSP2**

	Analytes	k_1	k_2	α	R_s	N_1	conditions
Ar-Ph	MDOPh-Ph	1.54	2.01	1.30	1.99	2404	ACN/H_2O=40/60
	4ClPh-Ph	1.44	1.84	1.27	1.72	2323	
	Ph-Ph	1.40	1.74	1.24	1.57	2327	
	4MOPh-Ph	4.32	5.07	1.17	2.19	4395	ACN/H_2O=30/70
	4NPh-Ph	4.61	5.23	1.14	1.63	4770	
	3ClPh-Ph	5.41	5.91	1.09	1.29	5014	
	3FPh-Ph	15.13	15.94	1.05	1.00	——	ACN/H_2O=20/80
Ar-Py	MDOPh-Py	8.59	9.52	1.11	1.50	4243	ACN/H_2O=20/80
	4ClPh-Py	9.21	10.07	1.09	1.32	4352	
	Ph-Py	6.21	6.75	1.09	1.17	4435	
	4MetPh-Py	10.47	11.16	1.07	0.96	4494	
	4MOPh-Py	6.98	7.39	1.06	0.74	3648	
	3ClPh-Py	8.34	8.48	1.02	≈0	——	
	3NPh-Py	5.99	0.00	1.00	0.00	——	
	4NPh-Py	7.07	0.00	1.00	0.00	——	
	4MetPh-2Py	9.05	9.92	1.10	1.48	5222	
Ar-OPr	2ClPh-OPr	11.51	12.38	1.08	1.16	5159	ACN/H_2O=10/90
	4ClPh-OPr	14.29	15.16	1.06	0.94	4934	
	4MOPh-OPr	11.12	11.76	1.06	0.85	4828	
	4MetPh-OPr	14.58	15.35	1.05	0.81	4891	

	4NPh-OPr	10.31	10.84	1.05	0.72	4158	
	Ph-OPr	8.49	8.82	1.04	0.49	3269	
	MDOPh-OPr	15.15	15.67	1.03	0.42	3106	
	3FPh-OPr	9.96	0.00	1.00	0.00	——	
	3ClPh-OPr	14.79	0.00	1.00	0.00	——	
	3NPh-OPr	6.37	0.00	1.00	0.00	——	
	4ClPh-OPr	7.92	8.56	1.08	1.17	——	
	2ClPh-OPr	7.31	7.94	1.09	1.14	4043	
	4NPh-OPr	6.09	6.58	1.08	0.99	3856	
	4MetPh-OPr	9.55	10.20	1.07	0.94	4279	
Ar-OPr	4MOPh-OPr	6.79	7.23	1.07	0.83	3930	MeOH/H_2O =30/70
	MDOPh-OPr	9.74	10.24	1.05	0.68	3827	
	Ph-OPr	5.10	5.26	1.03	0.20	——	
	3FPh-OPr	5.86	0.00	1.00	0.00	——	
	3ClPh-OPr	7.90	0.00	1.00	0.00	——	
	3NPh-OPr	4.14	0.00	1.00	0.00	——	

Conditions: flow rate = 0.6 mL·min^{-1}, 25 °C. ACN/H_2O=10/90

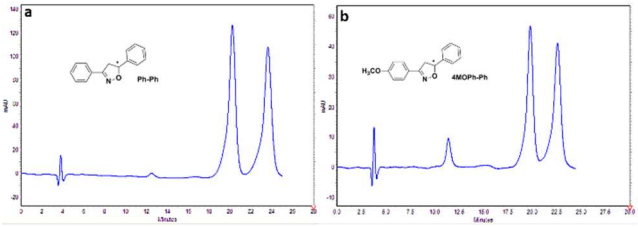

Fig.7 Representative chromatograms on **CSP2**. Conditions: ACN/H_2O (v/v) = 30/70; 25 °C; flow rate = 0.6 mL·min^{-1}

For Ar-Py and Ar-POr categories, only partial separation is achieved. Similar to MDOPh-Ph, MDOPh-Py affords the highest enantioselectivity amongst Ar-Py category. 4ClPh-Py was separated with a selectivity of 1.09 while that of 3ClPh-Py was almost zero. It was also found that no separations were with 3NPh-Py and 4NPh-Py, which indicates that the –NO_2 could decrease the enantioseparation under the studied separation conditions. It is interesting to find that 2ClPh-OPr exhibits better separation than 3ClPh-OPr on **CSP2**, which is different from the separation results on **CSP1**. This suggests that CSP linkages may bring about significant influence on the separation process.

3.3 Comparison of CSP1 and CSP2

Although **CSP1** and **CSP2** are both based on native CD, the minor difference in their structure may lead to remarkable distinct recognition ability. The linkage of **CSP1** can provide strong H-bonding sites, dipole-dipole and π-π interactions and **CSP2** affords electrostatic force on account of cationic heterocyclic ring on the linkage. Assisted by the strong electrostatic attraction, **CSP2** has been proven to provide superior separation efficiency than **CSP1** towards some aryl acidic racemates in our previous study. In this work, the comparisons of the two CSPs for enatioseparation of isoxazolines under same MP are plotted in Fig.8.

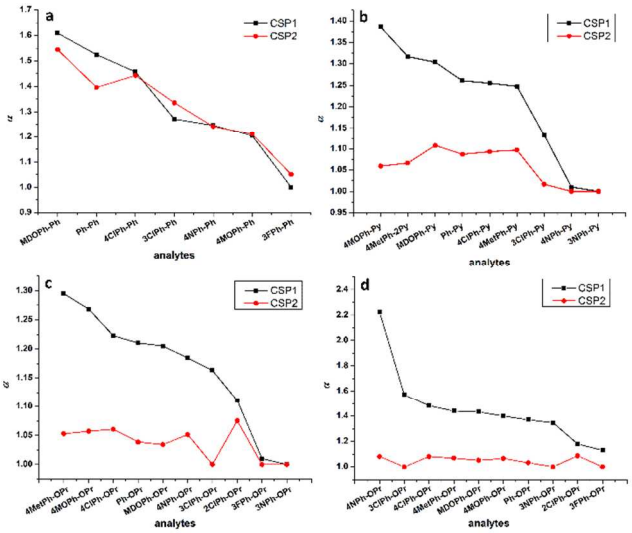

Fig.8 Comparison of the two CSPs for enatioseparation. Conditions : a) ACN/H_2O (v/v) =40/60, 25 °C; b) ACN/H_2O=40/60 (**CSP1**), ACN/H_2O=20/80 (**CSP2**), 25 °C; c) ACN/H_2O=30/70 (**CSP1**), ACN/H_2O=10/90 (**CSP2**), °C; d) MeOH/H_2O=50/50 (**CSP1**), MeOH/H_2O=30/70 (**CSP2**), 25 °C.

It can be seen clearly that **CSP1** affords higher enantioselectivity to most of the studied samples. On one hand, the higher surface CD loading of **CSP1** may contribute to the better separation; on the other hand, the abundant interaction sites on the linkage of **CSP1** must play important roles in the differentiation process after the formation of inclusion complex. As shown in Figure 8a, **CSP1** and **CSP2** affords close separation ability to the Ar-Ph category. The decreased enantioselectivity from MDOPh-Ph to 3FPh-Ph reveals a similar chiral differentiation mechanism for the Ar-Ph category on **CSP1** and **CSP2**. However, for Ar-Py category, **CSP1** exhibits much better separation capability than **CSP2** (Fig.8b). Hence, it's reasonable for us to believe that the triazole linkage on **CSP1** is more favorable for the separation process by supplying H-bonding and dipole-dipole interactions. This view is further convinced by the poorer separation of Ar-OPr category which bear more polar pyrrolidinone moieties on **CSP2** (Fig.8c). In addition, the different changing tendency of the selectivity from 4MOPh-Py to 3NPh-Py (Fig.8b) and from 4NPh-OPr to 3FPh-OPr (Fig.8c and 8d) suggests different separation mechanisms for Ar-Py and Ar-OPr categories on **CSP1** and **CSP2**.

In addition, the column efficiency of **CSP2** is higher than that of **CSP1** under the same separation conditions (Fig.S7, SI). The reason might be due to the lower CD loading and less interaction sites on the linkage.

3.4 Comparison with classic chiral column

In order to illustrate the good chiral differentiability of the current CSPs, a classic column – CYCLOBOND I *2000* (Purchased from Sigma Aldrich) was used for comparison by separation of a group of analytes (Ph-Ph, MDOPh-Ph, Ph-Py, 4ClPh-Py, Ph-OPr, 4ClPh-OPr) under the same conditions. It is interesting that CYCLOBOND I *2000* only afford enantioseparation ability toward 4ClPh-OPr with a selectivity of

1.16 (1.48 for **CSP1** and 1.08 for **CSP2**) under MeOH/H_2O=50/50, while most of the above analytes can be well enantioseperated on **CSP1** and **CSP2**. This result suggests that the well-defined CSP structures can afford superior separation to the studied isoxazoline derivatives.

4. Conclusions

"Click" derived thioether and triazole bridged native cyclodextrin chiral stationary phases were reported for well enantioseparation of isoxazoline derivatives for the first time. Aprotic acetonitrile is favorable for the enantioseparation of low polar Ar-Ph and Ar-Py, while protic methanol is a better organic modifier for high polar Ar-OPr. Isoxazoles with electron-rich groups can be resolved easier than those possessing electron-deficient parts. For most isoxazoline analytes, the separation of *p*-substitution on the 3-phenyl ring has advantages over that of *o*- and *m*-substitutions. In addition, CSP's resolution properties can be finely tuned by altering the linkages to introduce necessary effects such as H-bonding, π–π and dipole-dipole interactions. This work provides an effective and economic approach for the acquisition of optically pure substituted isoxazoline enantiomers.

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