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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 first 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 the enantioseparation of isoxazolines. Most of the studied isoxazolines were found to be well resolved (R_s > 1.5) under reversed phase mode, especially 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.

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1. Introduction

Compounds incorporating heterocyclic rings continue to attract considerable interest owing to their wide range of 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, *in 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] A 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 an isoxazole nucleus have been designed and synthesized. Our previous study described the synthesis of a variety of novel isoxazolines *via* a 1,3-dipolar cycloaddition reaction between mono-substituted alkenes and nitrile oxides.[15]

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

On a separate note, cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides that consist of several (6, 7, 8) glucose units and 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, they have received great interest in the area of separation. 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]

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In recent years, "click chemistry" has attracted 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 the Cu(I) catalytic 1,3-dipolar cycloaddition reaction and radical-based thiol–ene reaction, which have been widely used in the preparation of various stationary phases.[28–30] Great importance was placed on triazole-linked CD-CSPs that were prepared by 1,3-dipolar cycloaddition reaction owing to their simple synthesis, controllable structure, good stability and powerful resolving ability. Our group has employed a thiol–ene click reaction for constructing a novel cationic native CD-CSP for enhanced separation of acidic chiral compounds in HPLC.[31] Both the 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 the enantioseparation of 26 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-oxo-pyrrolidin-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 the 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), and 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 using a Milli-Q water purification system (Billerica, MA, USA). All the isoxazoline racemic pairs used were synthesized according to our previously 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 previous reports.[31,32] The detailed synthetic procedures are included in the ESI.† 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

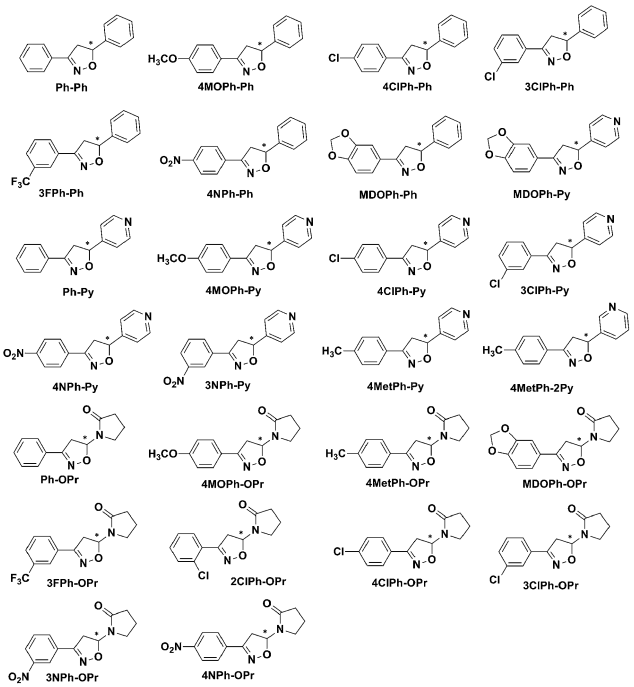

Fig. 1 Structures of isoxazoline derivatives.

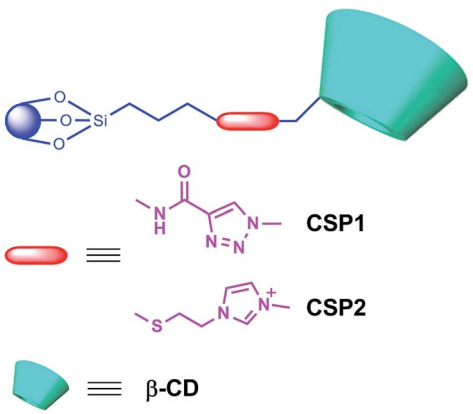

Fig. 2 Structures of triazole and thioether bridged CD-CSPs.

surface coverages of CD for **CSP1** and **CSP2** were calculated to be 0.85 μmol m^{-2} and 0.60 μmol m^{-2}, respectively, according to the following equation:

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

where S.A is the surface area of the silica; n_c is the carbon number; M_r is the molecular weight, and *C*% is from the elemental analysis results (ESI†).

The particle size distributions of the prepared CSPs are displayed in Fig. S3 (ESI†). The electron microscopy images of bare silica and the CSPs are illustrated in Fig. S4 (ESI†). Most of the particle sizes fall in the range from 3 to 7 μm, and the morphology of silica particles does not change much before and after reaction.

2.3 Column packing

The prepared CSPs were dispersed in MeOH followed by packing into a 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 a constant pressure of 5000 psi. The column efficiency for the studied analytes was calculated by the USP standard.

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 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 could 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 (ESI†). The encouraging results demonstrate that **CSP1** afforded powerful resolving ability towards the studied isoxazoline derivatives.

Table 1 Optimal separation results of isoxazolines on **CSP1**[a]

Analytes		k_1	k_2	α	R_s	N_1	Conditions
Ar-Ph	MDOPh-Ph	2.18	3.51	1.61	3.79	2163	ACN/H_2O = 40/60
	Ph-Ph	1.76	2.68	1.52	3.05	2122	
	4ClPh-Ph	1.95	2.84	1.46	2.78	2092	
	4MOPh-Ph	5.77	8.17	1.42	3.80	3822	ACN/H_2O = 30/70
	3ClPh-Ph	6.65	8.49	1.28	2.65	3668	
	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	ACN/H_2O = 30/70
	4MetPh-Py	3.72	4.69	1.26	1.97	2706	
	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	ACN/H_2O = 25/75
	4MOPh-OPr	1.85	2.33	1.26	1.63	2822	
	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	ACN/H_2O = 15/85
	2ClPh-OPr	9.62	10.75	1.12	1.32	4107	
	3NPh-OPr	3.79	4.14	1.09	0.79	3263	
Ar-OPr	4NPh-OPr	2.41	5.36	2.22	4.16	2157	MeOH/H_2O = 50/50
	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	
	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	

[a] Conditions: flow rate = 0.6 mL min^{-1}, 25 °C.

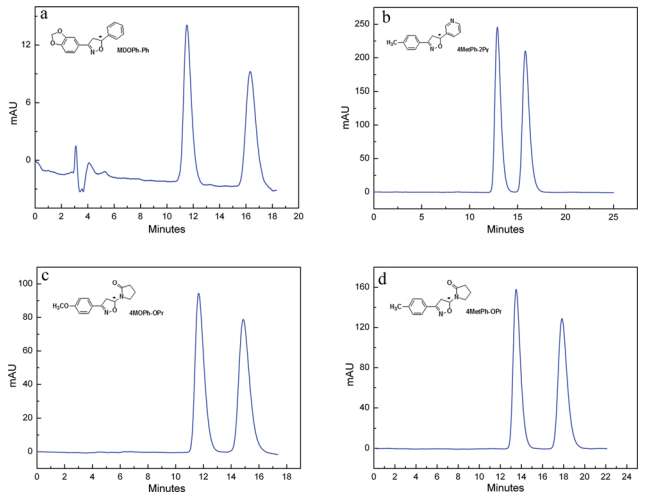

Fig. 3 Representative chromatograms on CSP1. Conditions: ACN/H_2O = 40/60 (a), ACN/H_2O = 30/70 (b), MeOH/H_2O = 50/50 (c and d); flow rate = 0.6 mL min^{-1}; 25 °C.

3.1.1 Effect of MP compositions on enantioseparation for CSP1. Since organic modifier plays key roles in the 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 the 5-position of the isoxazoline ring, the analytes can be classified into three categories: Ar-Ph (phenyl ring on the 5-position), Ar-Py (pyridine ring on the 5-position) and Ar-OPr (pyrrolidinone ring on the 5-position). To investigate the effect of the type of organic modifier, we chose several model analytes, Ph-Ph, 4MOPh-Ph, MDOPh-Ph, Ph-Py, 4MOPh-Py, 4MetPh-Py, Ph-OPr, 4ClPh-OPr, and 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[a]

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

[a] Conditions: flow rate = 0.6 mL min^{-1}, 25 °C; (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 a 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 transfer of 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 that 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 in resolving the more hydrophobic Ph-Ph and Ph-Py.

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

3.1.2 Enantioseparation of compounds in the Ar-Ph category. Based on the above-mentioned results, ACN/H_2O was chosen as MP for the separation of compounds in the 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 the 3- and 5-positions can enter the CD cavity first to form an inclusion complex, and 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

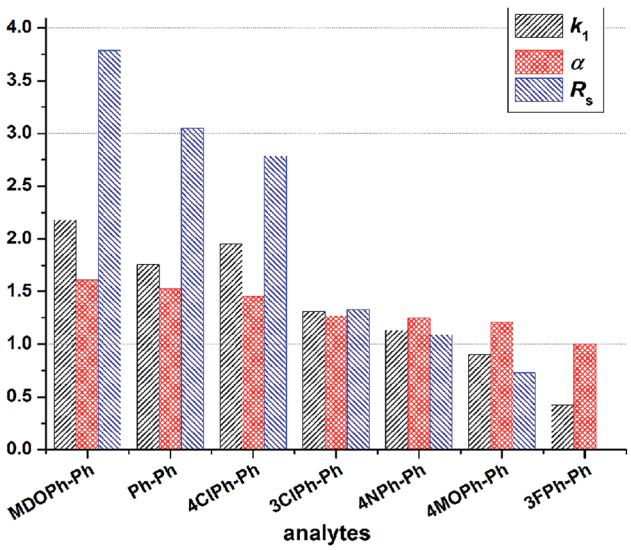

Fig. 4 Enantioseparation of Ar-Ph on CSP1. Conditions: flow rate = 0.6 mL min^{-1}, temperature = 25 °C.

separation, benefiting from the tight inclusion between the CD cavity and the enlarged hydrophobic part on the 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 the phenyl ring affords better inclusion formation ability than that at the *m*-position. The reduced retention and separation of 4NPh-Ph and 4MOPh-Ph suggests their loose inclusion with the CD cavity, which may 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 by 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 when 20% ACN was used (Table 1).

3.1.3 Enantioseparation of compounds in the Ar-Py category. Similar to the separation of compounds in the Ar-Ph category, substitute of $-NO_2$ on the phenyl ring strongly weakens the separation of compounds in the Ar-Py category, such as 4NPh-Py and 3NPh-Py, as shown in Fig. 5, and the 3,4-methylenedioxy group on MDOPh-Py is helpful for enhancing the chiral differentiation. In addition, 4ClPh-Py also gains much better resolution than 3ClPh-Py. However, the –OMe on the *p*-position strongly enhances the chiral recognition of Ar-Py (4MOPh-Py), which is different from compounds in the 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 a H-bond 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 support 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.

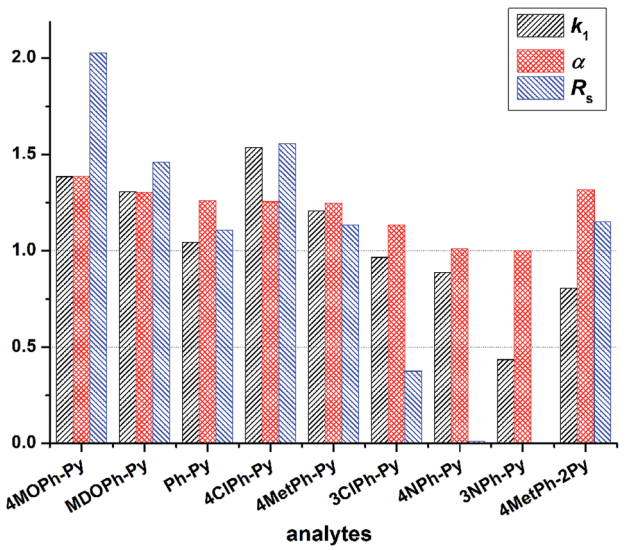

Fig. 5 Enantioseparation of Ar-Py on **CSP1**. Conditions: flow rate = 0.6 mL min^{-1}, temperature = 25 °C.

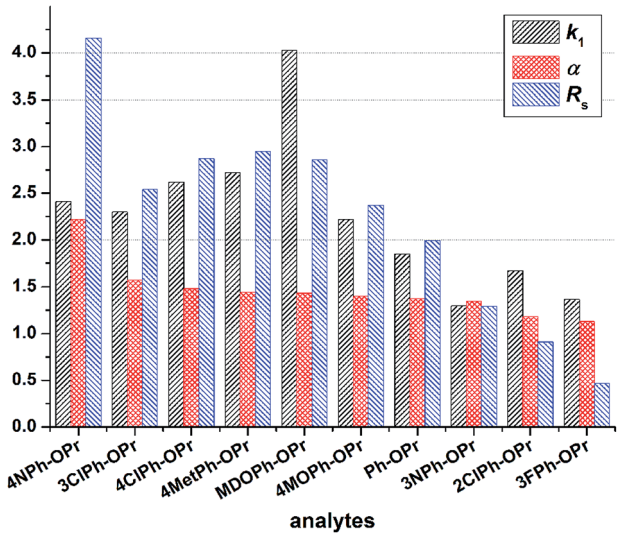

Fig. 6 Enantioseparation of Ar-OPr on **CSP1**. Conditions: flow rate = 0.6 mL min^{-1}, temperature = 25 °C.

3.1.4 Enantioseparation of compounds in the Ar-OPr category. The enantioseparation of compounds in the 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 an important role in the chiral differentiation process. The *p*-NO_2 phenyl ring of 4NPh-OPr may form the most favorable binding geometry with the CSP, hence showing the best enantioselectivity (α = 2.22) and resolution (R_s = 4.16), while the $-NO_2$ at the *m*-position decreases the separation remarkably. By a comparison of 4ClPh-OPr, 3ClPh-OPr, 2ClPh-OPr and Ph-OPr, it was found that the retention and resolution follows an order of 4ClPh > 3ClPh > Ph > 2ClPh, indicating that –Cl on the *p*- and *m*-positions favors the separation, while –Cl on the *o*-position attenuates the chiral differentiation.

Further study of 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.

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

CSP2 presents similar recognition behaviors as **CSP1**. Compounds in the Ar-Ph and Ar-Py categories can be better separated in the presence of ACN than MeOH, while the reverse

Table 3 Optimal separation results of isoxazolines on CSP2[a]

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	—	
Ar-OPr	4ClPh-OPr	7.92	8.56	1.08	1.17	—	MeOH/H_2O = 30/70
	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	
	4MOPh-OPr	6.79	7.23	1.07	0.83	3930	
	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	—	

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

situation was found for the separation of compounds in the 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. Greater enantioselectivity is achieved for 4ClPh-Ph than for 3ClPh-Ph, and 3FPh-Ph shows the poorest separation.

For compounds in the Ar-Py and Ar-POr categories, only partial separation is achieved. Similar to MDOPh-Ph, MDOPh-Py affords the highest enantioselectivity amongst compounds in the 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 achieved 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 have a significant influence on the separation process.

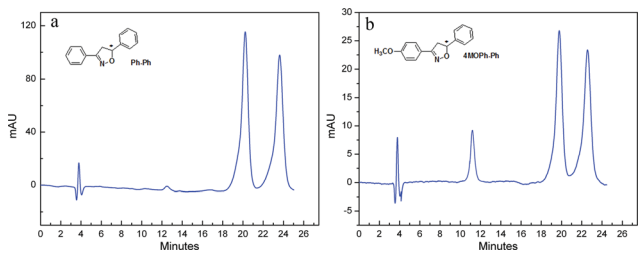

Fig. 7 Representative chromatograms on CSP2. Conditions: ACN/H_2O (v/v) = 30/70; 25 °C; flow rate = 0.6 mL min^{-1}.

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 remarkably distinct recognition abilities. The linkage of **CSP1** can provide strong H-bonding sites, dipole–dipole and π–π interactions, and **CSP2** affords an electrostatic force due to the 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

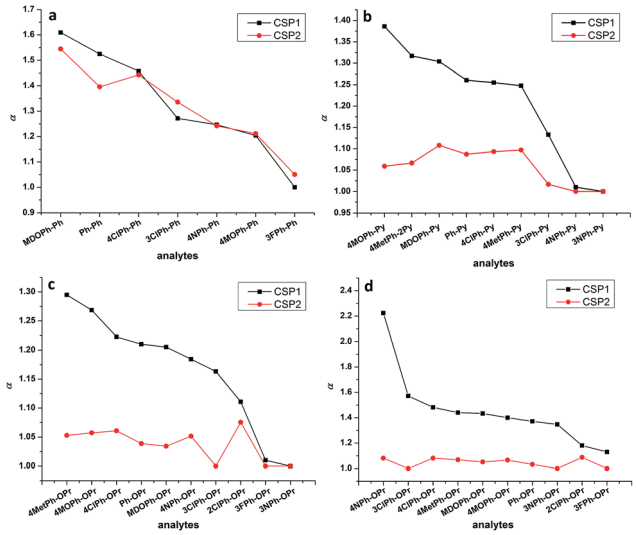

Fig. 8 Comparison of the two CSPs for enatioseparation. Conditions: (a) ACN/H_2O (v/v) = 40/60, 25 °C; (b) ACN/H_2O = 40/60 (**CSP1**), ACN/H_2O = 20/80 (**CSP2**), 25 °C; (c) ACN/H_2O = 30/70 (**CSP1**), ACN/H_2O = 10/90 (**CSP2**), °C; (d) MeOH/H_2O = 50/50 (**CSP1**), MeOH/H_2O = 30/70 (**CSP2**),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

racemates in our previous study. In this work, the comparisons of the two CSPs for the enatioseparation of isoxazolines with same MP are plotted in Fig. 8.

It can be seen clearly that **CSP1** affords higher enantioselectivity compared 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 an inclusion complex. As shown in Fig. 8a, **CSP1** and **CSP2** afford close separation ability for compounds in the Ar-Ph category. The decreased enantioselectivity from MDOPh-Ph to 3FPh-Ph reveals a similar chiral differentiation mechanism for compounds in the Ar-Ph category on **CSP1** and **CSP2**. However, for compounds in the Ar-Py category, **CSP1** exhibits a much better separation capability than **CSP2** (Fig. 8b). Hence, it is reasonable for us to believe that the triazole linkage on **CSP1** is more favorable for the separation process *via* the supply of H-bonding and dipole–dipole interactions. This view is further confirmed by the poorer separation of compounds in the Ar-OPr category, which bear more polar pyrrolidinone moieties on **CSP2** (Fig. 8c). In addition, the differences in the changing tendencies of the selectivity from 4MOPh-Py to 3NPh-Py (Fig. 8b) and from 4NPh-OPr to 3FPh-OPr (Fig. 8c and d) suggest different separation mechanisms for compounds in the 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, ESI†). The reason may be due to the lower CD loading and fewer 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 a comparison by the 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 afforded 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 of the studied isoxazoline derivatives.

4. Conclusions

For the first time, "click"-derived thioether and triazole bridged native cyclodextrin chiral stationary phases were reported to provide good enantioseparation of isoxazoline derivatives. Aprotic acetonitrile is favorable for the enantioseparation of low polar Ar-Ph and Ar-Py, while protic methanol is a better organic modifier for highly 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 the *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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References

1 C. Zhao and J. E. Casida, *J. Agric. Food Chem.*, 2014, **62**, 1019–1024.
2 C. J. A. Ribeiro, J. D. Amaral, C. M. P. Rodrigues, R. Moreira and M. M. M. Santos, *Bioorg. Med. Chem.*, 2014, **22**, 577–584.
3 A. Padmaja, C. Rajasekhar, S. Durgamma, B. Venkatesh and V. Padmavathi, *Med. Chem. Res.*, 2014, **23**, 1084–1098.
4 G. Lavanya, L. Mallikarjuna Reddy, V. Padmavathi and A. Padmaja, *Eur. J. Med. Chem.*, 2014, **73**, 187–194.
5 H. Juneja, D. Panchbhai, J. Sheikh, V. Ingle and T. Hadda, *Med. Chem. Res.*, 2014, **23**, 1537–1547.
6 C. Gan, Q. Lin, J. Cui, J. Feng, J. Guo, H. Liao and Y. Huang, *Steroids*, 2014, **79**, 37–43.
7 P. Fagone, K. Muthumani, K. Mangano, G. Magro, P. L. Meroni, J. J. Kim, N. Y. Sardesai, D. B. Weiner and F. Nicoletti, *Immunology*, 2014, DOI: 10.1111/imm.12267.
8 L. L. Fershtat, I. V. Ovchinnikov and N. N. Makhova, *Tetrahedron Lett.*, 2014, **55**, 2398–2400.
9 R. S. Chavan and A. P. Khadke, *Asian J. Chem.*, 2012, **24**, 2711–2716.

10 A. Kamimura, R. Takeuchi, K. Ikeda, T. Moriyama and M. Sumimoto, *J. Org. Chem.*, 2012, **77**, 2236–2245.
11 P. Sudhir, C. Rajashree and B. Ashok, *E-J. Chem.*, 2012, **9**, 1760–1772.
12 M. R. Barbachyn, G. J. Cleek, L. A. Dolak, S. A. Garmon, J. Morris, E. P. Seest, R. C. Thomas, D. S. Toops, W. Watt, D. G. Wishka, C. W. Ford, G. E. Zurenko, J. C. Hamel, R. D. Schaadt, D. Stapert, B. H. Yagi, W. J. Adams, J. M. Friis, J. G. Slatter, J. P. Sams, N. L. Oien, M. J. Zaya, L. C. Wienkers and M. A. Wynalda, *J. Med. Chem.*, 2002, **46**, 284–302.
13 A. Tamrakar, P. Tiwari, R. Ahmad, R. Kumar, V. Lakshmi, M. Srivastava and A. Srivastava, *Med. Chem. Res.*, 2008, **17**, 62–73.
14 G. Ahmad, P. K. Mishra, P. Gupta, P. P. Yadav, P. Tiwari, A. K. Tamrakar, A. K. Srivastava and R. Maurya, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 2139–2143.
15 Y. Gong, Y. Wang, W. T. Zhao and X. Y. Tang, *J. Chem. Res.*, 2013, 499–502.
16 P. Etayo and A. Vidal-Ferran, *Chem. Soc. Rev.*, 2013, **42**, 728–754.
17 S. Maddi, S. K. Yamsani, A. Devandla, G. Scriba and M. R. Yamsani, *Med. Chem. Res.*, 2013, **22**, 2403–2410.
18 S. Mohr, J. A. Weiss, J. Spreitz and M. G. Schmid, *J. Chromatogr. A*, 2012, **1269**, 352–359.
19 A. E. Schwaninger, M. R. Meyer and H. H. Maurer, *J. Chromatogr. A*, 2012, **1269**, 122–135.
20 D. W. Armstrong, T. J. Ward, R. D. Armstrong and T. E. Beesley, *Science*, 1986, **232**, 1132–1135.
21 Y. F. Poon, I. W. Muderawan and S. C. Ng, *J. Chromatogr. A*, 2006, **1101**, 185–197.
22 I. W. Muderawan, T.-T. Ong and S.-C. Ng, *J. Sep. Sci.*, 2006, **29**, 1849–1871.
23 Y. P. Zhang, Z. M. Guo, J. X. Ye, Q. Xu, X. M. Liang and A. W. Lei, *J. Chromatogr. A*, 2008, **1191**, 188–192.
24 Z. M. Guo, Y. Jin, T. Liang, Y. F. Liu, Q. Xu, X. M. Liang and A. W. Lei, *J. Chromatogr. A*, 2009, **1216**, 257–263.
25 Y. Wang, T. T. Ong, L. S. Li, T. T. Y. Tan and S. C. Ng, *J. Chromatogr. A*, 2009, **1216**, 2388–2393.
26 Y. Wang, Y. Xiao, T. T. Y. Tan and S. C. Ng, *Tetrahedron Lett.*, 2008, **49**, 5190–5191.
27 F. Ai, Y. Wang, H. Chen, Y. Yang, T. T. Y. Tan and S.-C. Ng, *Analyst*, 2013, **138**, 2289–2294.
28 P. Thirumurugan, D. Matosiuk and K. Jozwiak, *Chem. Rev.*, 2013, **113**, 4905–4979.
29 Y. Lv, Z. Lin and F. Svec, *Analyst*, 2012, **137**, 4114–4118.
30 A. Dondoni and A. Marra, *Chem. Soc. Rev.*, 2012, **41**, 573–586.
31 X. B. Yao, T. T. Y. Tan and Y. Wang, *J. Chromatogr. A*, 2014, **1326**, 80–88.
32 Y. Wang, H. Chen, Y. Xiao, N. Cheong Hengq, T. S. Oh, T. T. Y. Tan and N. Siu Choon, *Nat. Protoc.*, 2011, **6**, 935–942.