Synthesis and Chromatographic Properties of New β-Cyclodextrin Derivatives with α-Schiff Base Groups for HPLC

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Abstract: β-Cyclodextrin **1** was directly oxidized to the corresponding monoaldehyde **2** on their primary faces by cyclized 2-iodoxybenzoic acid(IBX) in DMSO, followed by the synthesis of β-cyclodextrin derivatives bearing Schiff-base group **3**. A new chiral stationary phase(BCDS **6**) was then prepared by immobilization of β-cyclodextrin derivative with α-Schiff base group onto the surface of sillica gel. A series of compounds with amino groups were readily separated using this CSP. Methanol and acetonitrile were tested as the mobile phase while the influence of temperature and the addition of aqueous triethylammonium acetate buffer to the mobile phase was also innvestigated. Ferrocene ligand with Schiff-based groups have been separated satisfactorilly on BCDS column.

Keyword: β-Cyclodextrin, Schiff base, ferrocene.

Cyclodextrins and their derivatives have played important roles in the diverse fields such as chiral separation, artifical enzymes, asymmetric synthesis and drug delivery[1]. It is necessary to design and prepare new cyclodextrin derivatives to expand their usage. Considering the good applications of Schiff bases in asymmetric catalysis[2], and its convenient introduction into cyclodextrins, we prepared the β-CDs derivatives with α-Schiff base group—a series of new CSPs. Here we report the practical synthesis of an examples of these compounds, which contain phenyl imino groups.

The synthetic route is outlined in **Scheme 1**. β-Cyclodextrin monoaldehyde **2** was oxidized with a mild and chemo-selective oxidizing agent, IBX[3,4]. Yields were routinely higher than 95%. The Dess-Martin periodinane(DMP), an another related oxidizing agent, was also tested. The result was not inspired because of the low efficiency and difficulty to be handled[5]. ^{1}HNMR (DMSO-d_6) showed that the intensity ratio of signals at δ 4.93 and δ4.83 is about 1:6. It indicated that the β-CD was mono-oxidized. Long reaction time and large excess of the oxidant were unfavorable, due to induce the formation of di- or trialdehydes. The method used for preparing the substances **3-6** is described in **Scheme 1.**

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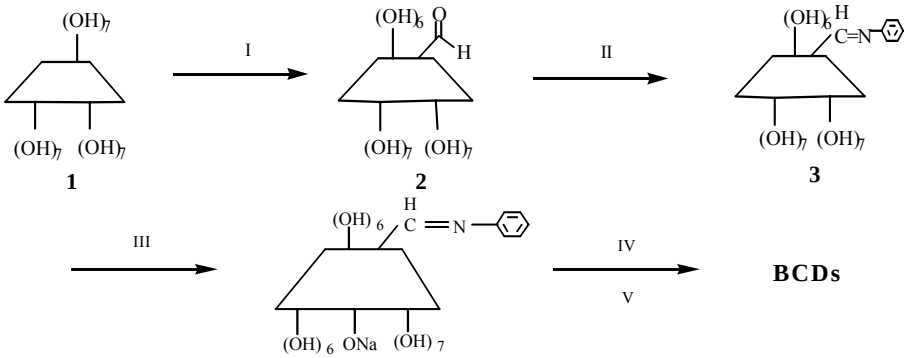
Scheme 1



Reaction Conditions: I: IBX, 30 °C, DMSO, 6 h; II: aniline, pyridine, 30°C, N_2, 4 d; III: NaH, DMF, 30 °C, N_2,1 h; IV: KH-560, DMF, 80 °C, N_2, 4 h; V: silica gel, DMF, 100 °C, N_2, 24 h;

Table 1 Effect of composition of mobile phase on the separation of the samples on BCDS

No.	compounds	MeOH/H_2O (v/v) 30:70		20:80		10:90		0:100	
		k^a	α^b	k^a	α^b	k^a	α^b	k^a	α^b
1	isopropylamine	0.23	1.00	0.34	1.00	0.53	1.00	0.80	1.00
2	2-methoxyaniline	0.46	2.00	1.95	5.74	2.64	4.98	2.20	2.75
3	aniline	0.64	1.39	2.67	1.37	4.20	1.59	4.80	2.18

k^a is capacity factor; α^b is separation factor; HPLC conditions:flow-rate, 0.6 mL/min; detection, 254 nm; silica gel: 5μm, 100Å

6-Imino-β-cyclodextrin **3** was prepared with β-cyclodextrin monoaldehydes **2** and aniline. The effects of the solvents were investigated, finally pyridine was found to be suitable as its good solubility and convenience to treatment. MS and ^{1}HNMR spectra of compound **3** showed the expected structure. BCDS was prepared according to the procedure reported in the literature[6]. Two methods for the preparation of the BCDS were tried, among them, 6-imino-β-cyclodextrin **3** were immobilized onto the silica gel directly to be more convenient to manipulate.

The BCDS was slurry-packed into a 150 mm×4.6 mm i.d. stainless-steel LC column. Triethylammonium acttate buffers(TEAA) were prepared from 1% aqueous trithylamine by addition of glacial acetic acid to the desired pH. The mobile phases comprising of triethylammonium acetate buffers and the appropriate amount of the organic modifier , were filtered through a membrane filter of 0.5 μm pore size and degassed *prior to* use. The flow-rate was 0.6 or 1.0 mL/min. The samples were dissolved into the acetonitrile and filtered through a filter of 0.45 μm size.

The compounds with amino group were used for determination of the efficiency of the BCDS column. **Table 1** shows that the variation of the retention behavior of the samples on the BCDS with the content of methanol in the methanol-water mobile phase. As the methanol content in the mobile phase increases, the value of the k decreases,

which suggests that the BCDS can behave as a reversed phase packing. **Table 2** shows that the retention ability increases slightly when the temperature rised to 50°C, which implied that temperature had little effect on separation in HPLC.

Table 2 Effect of different temperature on the separation of the samples on BCDS

No.	Compounds	Temperature(°C)					
		25			50		
		k^a	α^b	R_s	k^a	α^b	R^s
1	isopropylamine	0.24	1.00	-	0.18	1.00	-
2	2-methoxyaniline	1.20	5.00	2. 6	0.78	4.33	1. 8
3	aniline	2.10	1.67	1. 2	1.28	1.64	0. 9

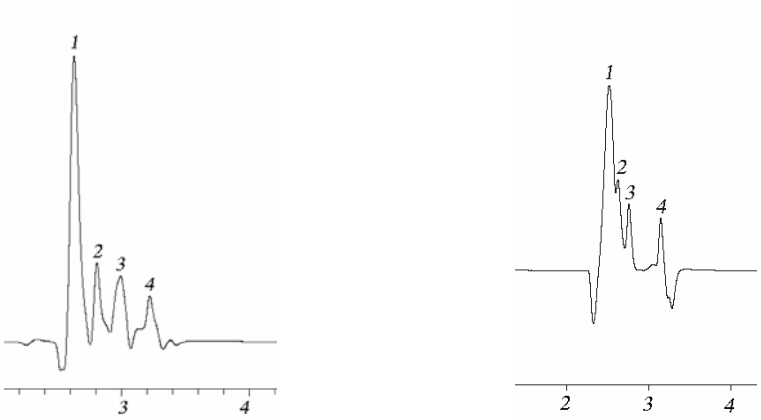
k^a is capacity factor; α^b is separation factor; HPLC conditions:flow-rate,1.0 mL/min; detection, 254 nm; mobile phase, methanol/water=10/90(v/v)

Table 3 Separation results for some positional isomers

No.	Compounds	Chromatographic data			Separation condition
		k^a	α^b	R_s	
1	*o*-toluidine	0.24	1.00	-	
2	*p*-toluidine	0.34	1.42	0. 8	I
3	*m*-toluidine	2.49	7.32	2. 9	
4	*p*-nitrotoluene	0.28	1.00	-	
5	*m*-nitrotoluene	2.38	8.50	1. 9	II
6	*o*-nitrotoluene	2.84	1.19	0. 6	

k^a: capacity factor; α^b: separation factor ; silica gel:5 μm, 100Å; HPLC conditions: flow-rate, 0.6 mL/min; detection, 254 nm; mobile phase, I: acetonitrile/water=10/90(v/v), II: acetonitrile/ water =20/80(v/v)

Figure 1 Chromatogram of imino samples on BCDS



(a) mobile phase: acetonitrile/water =10/90(v/v) flow-rate, 0.6 mL/min; detection, 254 nm

(b) mobile phase:acetonitrile/ TEEA = 10/90(v/v)pH=6.2, flow-rate, 0.6 mL/min; detection, 254 nm

1) N-*sec*-butyl-1-ferrocenyl-benzalimine; 2) N-isopropyl-1-ferrocenyl benzalimine;
3) N- phenyl -1-ferrocenyl-benzalimine; 4) N-1-phenylethyl-1-ferrocenyl benzalimine

The chromatographic performance of the column was evaluated using compounds with positional isomers, nitrotoluene and toluidine. **Table 3** gives the retention data of the samples obtained on the BCDS in different conditions.

Chiral ferrocene ligands with Schiff-based groups have been studied by many chemists[7] because of its good application in asymmetric catalysis and convenience to synthesize. To our knowledge, few work were reported on the separation of the Schiff bases. In our case, Schiff bases have a good separation on the BCDS column prepared, which can be demonstrated by the high column efficiency (N=119322 P/m) (**Figure 1, a**) and resolutions (R_s=1.706,1.561,1.605) (**Figure 1, a**). It was found that the buffer mobile phase was not fit for the separation. The reason may be that the samples were dissolved in acetonitrile, which was more powerful in the washing intensity than the mobile phase.

In order to test the enantioselectivity to chiral compounds on this new derivatives stationary phases for HPLC, study on the separation of the chiral compouds was carried out.

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