

Chiral Separation Abilities of Homogeneously Synthesized Cellulose 3,5-Dimethylphenylcarbamates: Influences of Degree of Substitution and Molecular Weight*

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Abstract A series of cellulose 3,5-dimethylphenylcarbamates (CDMPCs) with different degrees of substitution (DS) and degrees of polymerization (DP) were homogeneously synthesized in 1-allyl-3-methylimidazolium chloride (AmimCl). Then, the CDMPCs were coated on silica gel and used as chiral stationary phases (CSPs), and their chiral recognition abilities for seven racemates were evaluated by high performance liquid chromatography. The results showed that DS and DP of CDMPCs had a great influence on chiral recognition abilities of the CSPs. The CSPs with the DS ≈ 1 gives a low chiral recognition to most racemates. On the contrast, the CSPs with the DS ≥ 2 exhibited high chiral separation abilities. For example, six racemates could be separated on the CSP with CDMPC of DS ≈ 2 (CSP-2). Especially, for the enantioseparation of 1-(2-naphthyl) ethanol and Tröger's base, CSP-2 gave the highest separation ability in all of CSPs. On the other hand, when the DP of cellulose was in a range from 39 to 220, the chiral separation abilities of CDMPCs increased as the DP increased. This work demonstrates that the structure of cellulose esters such as DS and DP has important effect on their chiral separation ability, and therefore provides a practical method to design and prepare desirable CSPs for different racemates.

Keywords: Cellulose phenylcarbamate; Ionic liquid; Degree of substitution; Degree of polymerization; Chiral separation.

INTRODUCTION

As a practical method for both determining optical purity and obtaining optically pure enantiomers, using chiral stationary phases (CSPs) in high performance liquid chromatography (HPLC) to directly separate racemates is attractive, particularly in the pharmaceutical industry. Among commercial CSPs, phenylcarbamates and benzoates of cellulose and amylose exhibit high chiral separation abilities, and nearly 90% of chiral compounds can be successfully resolved^[1, 2]. However, because cellulose is neither meltable nor soluble in conventional solvents, the traditional method to prepare phenylcarbamates and benzoates of cellulose begins with a heterogeneous reaction and only fully-substituted cellulose derivatives are achieved^[3–6]. Under the heterogeneous reaction, it is impossible to get partially substituted products and control the distribution of substituents directly^[7–9]. Actually, the structure of cellulose derivatives, such as degree of substitution (DS),

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degree of polymerization (DP) and distribution of substituents on the cellulose, has significant impact on their helical structure and local polarity, and accordingly on their recognition abilities. Toga *et al.* studied the chiral recognition ability of partially substituted cellulose benzoates with DS from 2.1 to 2.8, which were prepared by aminolysis of cellulose tribenzoate, and indicated that some racemates were resolved only by partially substituted cellulose benzoates^[10]. Enomoto *et al.* prepared some amylose based-CSPs with amylose of different DP and found that in a range of DP from 7 to 120, the resolving power of CSPs tends to increase as the DP of amylose increases^[11].

Homogeneous derivatization of cellulose in appropriate media, the alternative procedure for heterogeneous process, has drawn much attention, because it can excellently control the DS value, distribution and uniformity of functional groups along cellulose chains, and avoid many problems associated with the heterogeneous reactions, such as non-uniform distribution of substituents, side reactions and time consumption. Recently, ionic liquids (ILs) with certain structure were reported to dissolve cellulose excellently, providing a new and versatile platform for the homogeneous synthesis of cellulose derivatives^[12, 13]. A variety of cellulose esters were prepared by using ILs^[14–18]. The homogeneous synthesis in ILs not only provides opportunities to control the structure of cellulose derivatives, but also gives a high efficiency and yield without a catalyst or protecting process commonly.

In this work, *via* a homogeneous synthesis route in an ionic liquid 1-allyl-3-methylimidazolium chloride (AmimCl), a series of cellulose 3,5-dimethylphenylcarbamates (CDMPCs) with different structures were prepared. Then, the influence of the DS and DP of CDMPCs on their chiral recognition abilities was investigated.

EXPERIMENTAL

Materials and Apparatus

Ionic liquid AmimCl was synthesized according to our previous work^[19]. 3,5-Dimethylphenyl isocyanate was purchased from Maya Reagent Company. 3-Aminopropyltriethoxysilane was purchased from TCI. The racemates: (1) 2-phenylcyclohexanone, (2) Tröger's base, (3) D,L-sec-phenethyl alcohol, (4) flavanone, (5) 1-(2-naphthyl)ethanol, (6) cobalt(III) acetylacetonate and (7) *trans*-stilbene oxide were purchased from Sigma Aldrich. Their structures are shown in Fig. 1. All other chemicals were obtained from Beijing Chemical Reagent Company, China. Porous sphere silica gel with a mean particle size of 5.8 μm and a mean pore diameters of 26 nm was purchased from Beijing Greenherbs Science and Technology Development Company. It was treated with 3-aminopropyltriethoxysilane in toluene to functionalized as 3-aminopropyl silica before use.

Microcrystalline cellulose (MCC) and cotton pulp with degree of polymerization (DP) of 220 and 650 were dried under vacuum at 80 °C for 24 h prior to use. Three cellulose samples with lower DPs (39, 70 and 140) were prepared by the hydrolysis degradation of MCC in H_3PO_4 ^[20]. The DPs of all cellulose samples were determined by the Ubbelohde viscometer method according to GB 5888-86^[21].

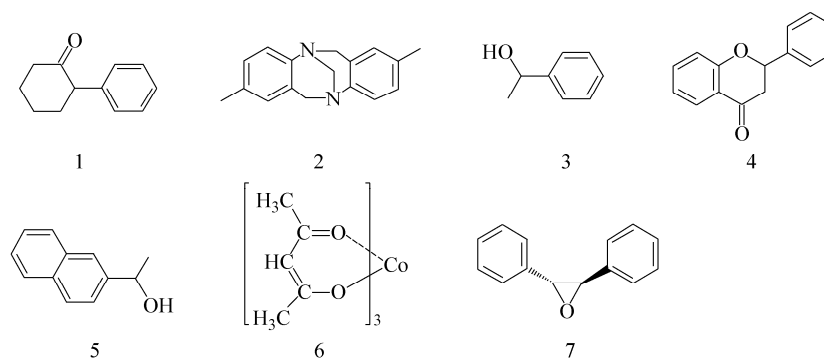


Fig. 1 Molecular structures of racemates

^1H -NMR spectra were acquired on a Bruker NMR spectrometers AV400 with 16 scans at room temperature in DMSO-d_6 . A few drops of trifluoroacetic acid- d_1 were added to shift active hydrogens to lower field area.

The degree of substitution (DS) of cellulose derivatives was calculated from ^1H -NMR by the following equation^[22]:

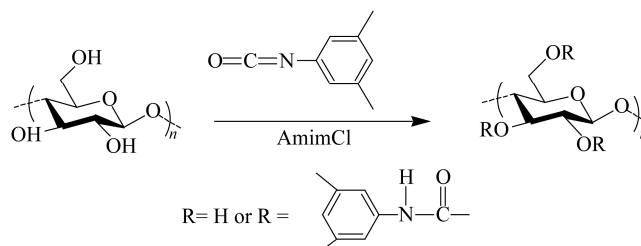
$$\text{DS} = 7I_{\text{phenyl}} / nI_{\text{AGU}} \quad (1)$$

I_{phenyl} , peak integral of phenyl protons; I_{AGU} , peak integral of protons of anhydroglucose unit (AGU); n , the number of protons on the benzene ring of 3,5-dimethylphenyl isocyanate.

Chromatography was performed at 25 °C using a Waters e2695 liquid chromatograph equipped with an ultraviolet detector (Waters UV-2489) and an Empower workstation.

Synthesis of Cellulose 3,5-Dimethylphenylcarbamates

Cellulose 3,5-dimethylphenylcarbamates were synthesized homogeneously in AmimCl, according to Scheme 1. The dried cellulose was added to a certain amount of AmimCl in a three-necked flask. The mixture of cellulose/AmimCl was mechanically stirred at 80 °C for 2 h, yielding a homogeneous solution with 4.0 wt% of cellulose concentration. At a given temperature, 3,5-dimethylphenyl isocyanate was added into cellulose/AmimCl solutions under vigorous stirring. After the required time, the resultant products were isolated as methanol-insoluble fractions, filtered and washed three times with methanol. They were redissolved in DMSO, precipitated again and thoroughly washed with methanol. Finally, these products were dried under vacuum at 80 °C.



Scheme 1 The synthetic route of cellulose 3,5-dimethylphenylcarbamate

Preparation of CSPs with Cellulose 3,5-Dimethylphenylcarbamates and Packing Column

3-Aminopropyl silica gel (APS, 3.0 g) was suspended in tetrahydrofuran (THF, 20 mL). Then, the cellulose 3,5-dimethylphenylcarbamate (0.53 g)/THF (30 mL) solution was added dropwise to the APS/THF slurry at a rate of 0.03 mL·min⁻¹. The suspension was stirred overnight, and then the solvent was slowly removed in a rotary evaporator at room temperature. Finally, the CSPs were dried under vacuum at 50 °C.

Packing CSPs were suspended in a hexane/2-propanol mixture (70:30, *V/V*) and packed into 250 mm × 4.6 mm (inner diameter) columns by using the stirred slurry method. Packing pressure was 40 MPa. Hexane/2-propanol (90:10, *V/V*) was employed as the displacing solvent.

RESULTS AND DISCUSSION

Homogeneous Synthesis of Cellulose 3,5-Dimethylphenylcarbamates in AmimCl

Homogeneous synthesis of cellulose 3,5-dimethylphenylcarbamates was carried out in AmimCl without any catalysts. The ^1H -NMR spectra of CDMPCs with DS = 1.06, 1.99 and 3.0 are provided in Fig. 2. The peaks in the range of δ = 9.0–9.3, 6.1–7.2 and 1.8–2.4 are attributed to the amino protons, aromatic protons and methyl protons of 3,5-dimethylphenylcarbamate, respectively. The peaks in the range of δ = 2.8–5.5 are attributed to the protons of cellulose backbone. Those peaks prove that the carbanilation of cellulose was achieved successfully.

The DS values of CDMPCs in a wide range (from 0.27 to 3.0) could be regulated by controlling the reaction conditions, such as temperature, reaction time and molar ratio of 3,5-dimethylphenyl isocyanates/AGU. The detailed experimental conditions and results are presented in Table 1. It is clear that the reaction was accelerated as the increase of reaction temperature. Under the conditions of a molar ratio of 3:1 and reaction time of 2 h, the

increase of reaction temperature from 60 °C to 80 °C, and 100 °C led to an increase in the DS of the products from 0.52 to 1.17 and 1.72, respectively. The DS of the products increased as reaction time prolonged. With a molar ratio of 3:1 at 80 °C, an increase of reaction time from 0.5 h to 1.0, 1.5 and 2.0 h brought about an increase in DS from 0.47 to 0.83, 1.02 and 1.17, respectively. In addition, as the increase of the molar ratio of 3,5-dimethylphenyl isocyanate/AGU from 3:1 to 5:1, the DS of the products increased from 1.53 to 1.74. Furthermore, the DP of cellulose had a negligible impact on the DS of CDMPCs. At the same reaction conditions, five CDMPCs with DS = 3.0 were obtained from different celluloses with DP of 39–650.

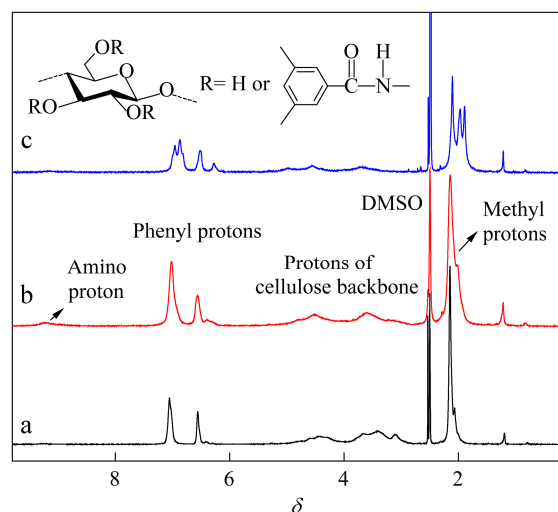


Fig. 2 ^1H -NMR spectra of cellulose 3,5-dimethylphenylcarbamate sample A5 with DS = 1.02 (spectrum a), sample A12 with DS = 1.99 (spectrum b) and sample A15 with DS = 3.0 (spectrum c)

Table 1. Cellulose 3,5-dimethylphenylcarbamates prepared homogeneously in AmimCl under various reaction conditions

Sample	Cellulose		Molar ratio of 3,5-dimethylphenyl isocyanate/AGU	T (°C)	Time (h)	DS ^a
	Type	DP				
A1	MCC	220	3:1	60	1.0	0.27
A2	MCC	220	3:1	60	2.0	0.52
A3	MCC	220	3:1	80	0.5	0.47
A4	MCC	220	3:1	80	1.0	0.83
A5	MCC	220	3:1	80	1.5	1.02
A6	MCC	220	3:1	80	2.0	1.17
A7	MCC	220	3:1	100	0.5	1.12
A8	MCC	220	3:1	100	1.0	1.53
A9	MCC	220	3:1	100	2.0	1.72
A10	MCC	220	5:1	100	0.5	1.16
A11	MCC	220	5:1	100	1.0	1.74
A12	MCC	220	5:1	100	1.7	1.99
A13	MCC	220	5:1	100	2.0	2.14
A14	MCC	220	5:1	100	4.0	2.79
A15	MCC	220	5:1	100	8.0	3.0
B1	Degraded cellulose 1	140	5:1	100	8.0	3.0
B2	Degraded cellulose 2	70	5:1	100	8.0	3.0
B3	Degraded cellulose 3	39	5:1	100	8.0	3.0
C	Cotton pulp	650	5:1	100	8.0	3.0

^a DS determined by ^1H -NMR spectra

Effect of the DS of CDMPCs on Enantioseparation of the CSPs

After coating cellulose 3,5-dimethylphenylcarbamates with DS = 1.02, 1.99 and 3.0 on silica gel, three CSPs were obtained and marked as CSP-1, CSP-2 and CSP-3. Subsequently, the chiral recognition abilities of the obtained

CSPs were evaluated using HPLC with seven racemates. The mobile phases were *n*-hexane/2-propanol mixture (98:2, *V/V*) with a flow rate of 1 mL·min⁻¹. The dead time (*t*₀) was determined using 1,3,5-tri-*tert*-butylbenzene as the unretained compound. UV detection was carried out at 254 nm. The results are summarized in Table 2. It is clear that those CSPs exhibit different chiral separation abilities, which depend markedly on the DS of CDMPCs. CSP-1 gives a low chiral recognition. Only three racemates can be separated partially on CSP-1. As DS increases to 1.99, CSP-2 shows a high chiral recognition. Most of the racemates, six racemates, can be separated on CSP-2. When the DS increase to 3.0 further, the corresponding CSP-3 also shows a high chiral recognition. Six racemates can be separated on CSP-3. It is worth noting that racemates separated on CSP-3 are similar but not identical ones on CSP-2, indicating the different chiral separation abilities of CSP-2 and CSP-3. In addition, the chiral discrimination abilities of CDMPCs are not proportional to DS. For example, for the separation of Tröger base and flavanone, the chiral separation ability of CSP-3 is higher than CSP-2, as shown in Figs. 3(a) and 3(b). However, for the separation of 1-(2-naphthyl) ethanol and cobalt(III) acetylacetonate, the chiral separation ability of CSP-2 is better than CSP-3, as shown in Figs. 3(c) and 3(d). Thus, the DS of CDMPCs had a great influence on chiral recognition abilities of the CSPs. For separating different racemates, the CSPs of CDMPCs with suitable DS should be chosen.

Table 2. Chromatographic resolution of racemates on CSPs coated with different DS of CDMPCs

Racemates	CSP-1			CSP-2			CSP-3		
	<i>k'</i> ₁ ^a	α ^b	<i>R</i> _s ^c	<i>k'</i> ₁	α	<i>R</i> _s	<i>k'</i> ₁	α	<i>R</i> _s
1	1.73	~1	—	1.89	1.08	1.29	1.88	1.22	1.98
2	1.83	1.07	1.02	1.55	1.20	3.05	1.27	1.48	3.48
3	1.03	~1	—	3.76	1.05	1.14	3.58	~1	—
4	2.30	~1	—	2.32	~1	—	2.13	1.13	1.14
5	1.45	1.05	0.81	7.74	1.12	3.47	8.11	1.06	0.89
6	0.78	1.05	0.53	2.55	1.19	2.79	1.75	1.11	1.15
7	0.52	~1	—	1.89	1.08	1.29	1.85	1.51	0.88

^a Capacity factor of the first eluted enantiomer estimated as (*t*₁ - *t*₀)/*t*₀;

^b Separation factor estimated as *k'*₂/*k'*₁;

^c Resolution estimated as 1.18(*t*₂ - *t*₁)/(*w*₁ + *w*₂), *t*: the retention time and *w*: the half-height peak width

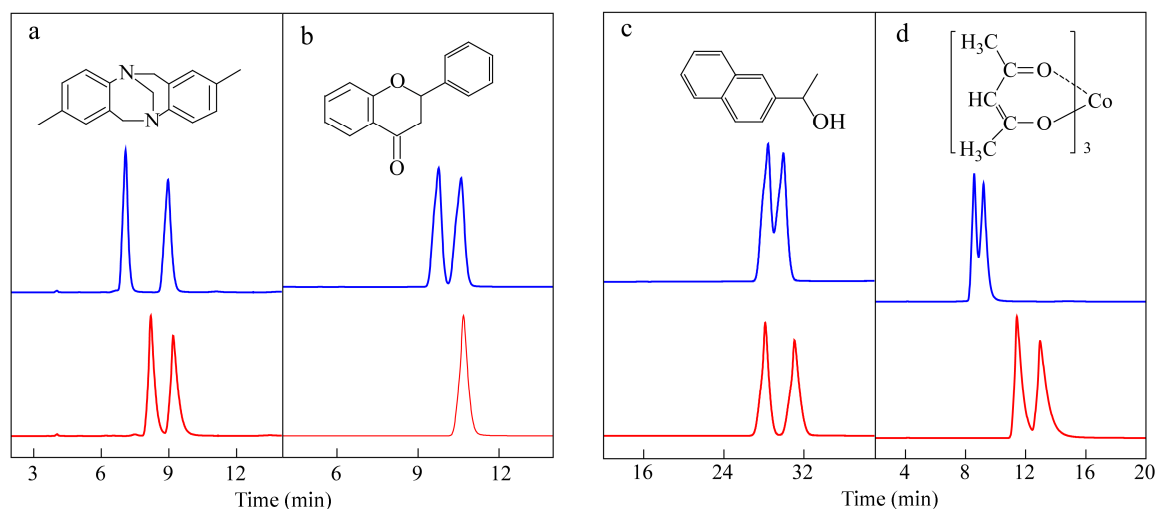


Fig. 3 Chromatographic resolution of (a) Tröger base, (b) flavanone, (c) 1-(2-naphthyl) ethanol and (d) cobalt(III) acetylacetonate on CSP-2 and CSP-3, respectively (CSP-2 and CSP-3 from the bottom up)

Effect of the DP of CDMPCs on Enantioseparation of the CSPs

CSPs prepared by coating cellulose tri(3,5-dimethylphenylcarbamates) with different DPs, were marked as CSP³⁹, CSP⁷⁰, CSP¹⁴⁰, CSP²²⁰ and CSP⁶⁵⁰, respectively. The effect of the DP of CDMPCs on the resolution of racemates

is shown in Fig. 4. As the DP increases from 39 to 140, the capacity factor k'_1 values of all racemates increase rapidly. When the DP increases further, the k'_1 values show a negligible change. The increase of k'_1 suggests that the racemates need more time to be eluted from HPLC columns. That is to say, the interaction time between racemates and CSPs prolongs. In general, the increase of DP of CDMPCs helps to increase the chiral discrimination ability of the CSPs. As shown in Fig. 4(b), for most of racemates, *i.e.* 1, 2, 5, 6 and 7, the chiral separation abilities of CSPs increase as the increase of the DP of CDMPCs. When the DP is in a range of 140–220, the chiral discrimination abilities of the corresponding CSPs are the best. For example, for the separation of flavanone (Fig. 5a) and cobalt(III) acetylacetonate (Fig. 5b), the chiral separation ability of CSP²²⁰ is obviously higher than CSP⁷⁰. However, too high DP will cause a poor dispersibility of the corresponding CSPs in *n*-hexane/2-propanol mixture, for example CSP⁶⁵⁰. So, CSP⁶⁵⁰ could not be tested in HPLC. In conclusion, the appropriate DP of CDMPCs (DP = 140–220) is beneficial to the chiral separation ability of CSPs.

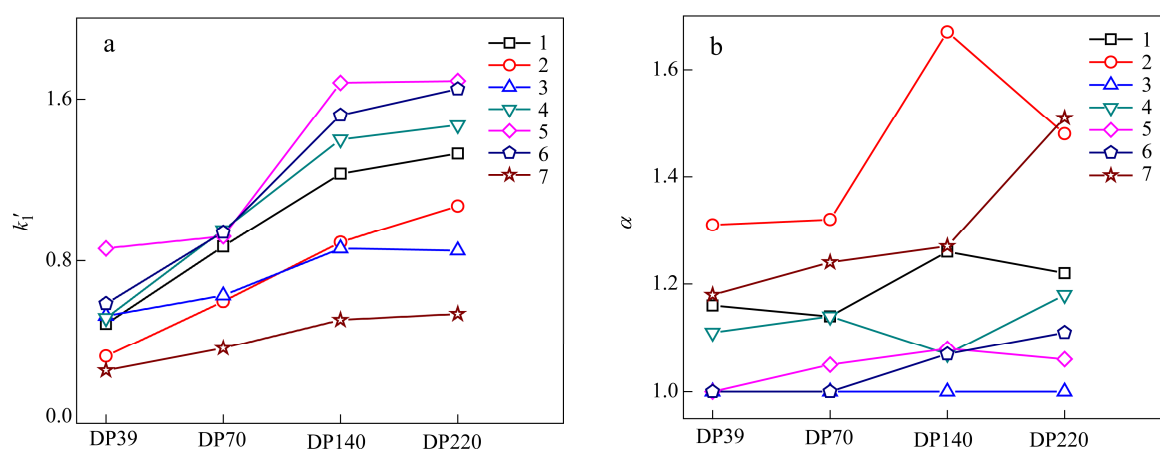


Fig. 4 Chromatographic resolution of seven racemates on CSPs coated with different DP of CDMPCs

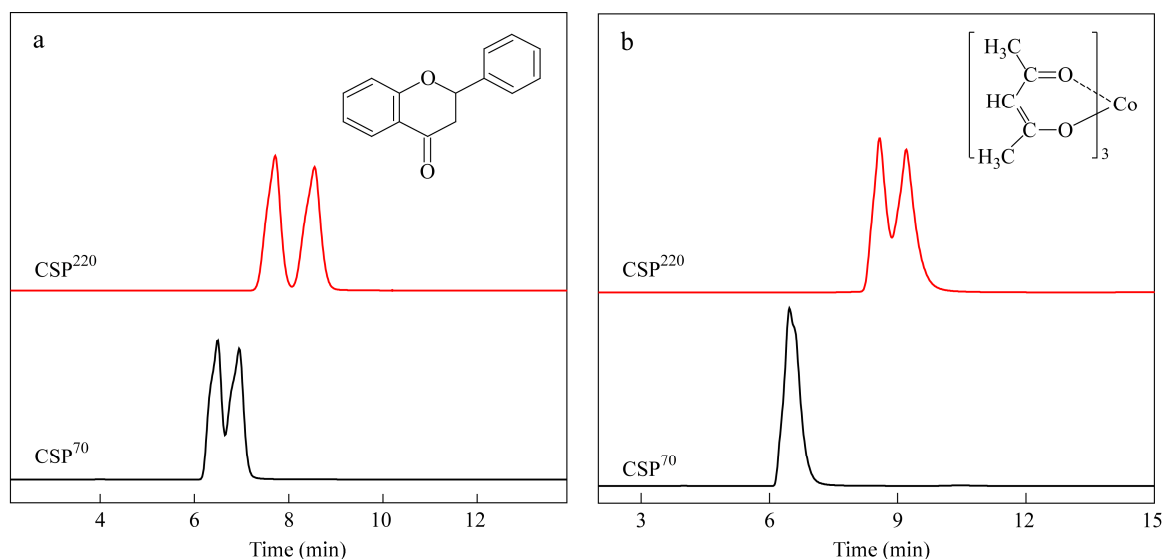


Fig. 5 Chromatographic resolution of (a) flavanone and (b) cobalt(III) acetylacetonate on CSP⁷⁰ and CSP²²⁰, respectively

CONCLUSIONS

Under mild conditions, the homogeneous carbanilation of cellulose with 3,5-dimethylphenyl isocyanates was successfully carried out in AmimCl without any catalysts. The DS of the products could be well controlled by

altering reaction temperature, reaction time, and molar ratio of 3,5-dimethylphenyl isocyanates/AGU. Subsequently, by taking advantage of the synthesis result, cellulose 3,5-dimethylphenyl carbamates with DS $\approx$ 1, 2, 3 and DP in a range of 39–650 were successfully prepared and coated on silica gel. The obtained CSPs were employed in chiral separation of seven racemates. The DS and DP of CDMPC have a marked effect on the chiral recognition abilities of CSPs. For some racemates, *e.g.* 1-(2-naphthyl) ethanol and cobalt(III) acetylacetonate, CSP with CDMPC of DS $\approx$ 2 had better chiral recognition abilities than fully substituted ones. When the DP was in a range of 140–220, the chiral discrimination abilities of the corresponding CSPs were the best. Our results preliminarily illuminate the structure-property relationship of CDMPC, which can be used to prepare suitable CSPs for different racemates.

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