Screening Approach for Chiral Separation of β-Aminoketones by HPLC on Various Polysaccharide-Based Chiral Stationary Phases

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ABSTRACT Nine β-aminoketones were synthesized via Mannich reaction when benzaldehyde was condensed with some primary amines and acetophenone. The purified compounds were identified by using spectroscopic methods. The enantiomeric separation of these derivatives was carried out by high-performance liquid chromatography (HPLC) using several coated and immobilized polysaccharide stationary phases, namely, Chiralcel® OD-H, Chiralcel® OD, Chiralcel® OJ, Chiralpak® AD, Chiralpak® IA, and Chiralpak® IB using different mobile phases composed of *n*-hexane and alcohol mixed in various ratios or pure ethanol or isopropanol. The retention behavior and selectivity of these chiral stationary phases were examined in isocratic normal phase mode. The results indicate that cellulose derivatives have higher enantioselectivity than amylose derivatives for the separation of racemic β-amino ketones. *Chirality 00:000–000, 2015.* © 2015 Wiley Periodicals, Inc.

KEY WORDS: β-aminoketones; chiral recognition mechanisms; chiral separation; enantioselectivity; Mannich reaction; polysaccharide-based chiral stationary phases

Multicomponent reactions (MCRs) have received significant research interest from chemical and medicinal communities. The Mannich reaction is one of the most-studied MCRs, discovered in 1912;[1–3] the product is an imino analog of the aldol condensation.[4] It is one of the most important carbon–carbon bond formation reactions in organic synthesis,[5–14] in which an aminomethyl group is introduced to the α position of a carbonyl function.[3,15] In the classical condensation, a single carbon atom is attached to an active methylene or methine group, using formaldehyde and an amine, to generate β-amino carbonyl compounds (Mannich bases), which are highly useful chiral building blocks[16,17] and important intermediates for the synthesis of various pharmaceutical and natural product synthesis including β-amino alcohols, β-amino acids, and β-lactams.[18]

The reaction has been extensively studied by organic chemists and has been the subject of numerous reviews. Kulkarni et al.[13] published an improved method consisting of a one-pot Mannich reaction of aromatic ketones, aromatic aldehydes, and aromatic amines using calcium chloride as catalyst in ethanol. The advantages of this new method are a short reaction time, high yield, easy workup, convenience, low cost, and eco-friendly protocol.[13]

The separation of chiral compounds has been of great importance, particularly in the pharmaceutical industry.[19] This interest is due to the different pharmacokinetic characteristics and pharmacological activities of each enantiomer in a racemic drug.[19] Chiral separation by high-performance liquid chromatography (HPLC) using a chiral stationary phase (CSP) is one of the most efficient methods for separating enantiomers, not only on an analytical scale, but also on a preparative scale.[19] In chiral liquid chromatography, polysaccharide-based CSPs are the most popular, among many chiral stationary phases. The acetate ester, benzoate ester, or phenylcarbamate derivatives of cellulose and amylose have shown broad enantioselectivity.[20] They are not only effective under normal-phase conditions, but also under reversed-phase conditions.[21–27]

In continuation of our work related to the chiral separation of several flavanone derivatives,[28–30] the present investigation describes the chiral HPLC separation of series of racemic β-amino ketones synthesized by Mannich reaction on several cellulose and amylose chiral selectors

MATERIALS AND METHODS

Instrumentation and Chromatographic Conditions

The enantioseparations were performed on an HPLC SHIMADZU LC 20-Asystem equipped with a DGU degasser, Shimadzu® LC 20 AD LC pump, a Rheodyne injector with 20 μl sample loop, and a UV detector Shimadzu® SPD-20 A (Kyoto, Japan).

The columns used in this study were purchased from Chiral Technologies Europe (Illkirch, France) with the following dimensions: Chiralcel® OD-H (150 × 4.6.mm I.D, 5 μm particle size), Chiralcel® OD, Chiralcel® OJ (250 × 4.6 mm I.D., 10 μm particle size), Chiralpak® AD (250 × 4.6. I.D. mm, 10μm particle size), Chiralpak® IA and Chiralpak® IB (150 × 4.6 mm I.D., 5 μm particle size).

The mobile phase for LC was filtered through a Millipore membrane filter (Bedford, MA; 0.5 μm) and degassed before use. Different mobile phase systems were investigated in this study that consisted of hexane and alcohol (ethanol or isopropanol) in different ratios (90:10 v/v and 95:5 v/v) or 100% ethanol or isopropanol. The chromatographic runs were performed at room temperature, ~25°C. The injection volume was 10 μL of a 1-mg sample solution dissolved in 1 mL of isopropanol.

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All solvents used were HPLC grade and purchased from Sigma-Aldrich (Lyon, France). All the starting reagents and solvents used in synthesis were of standard analytical grade from

Fluka (Buchs, Switzerland), Merck (darmstadt, Germany), and Sigma Aldrich, Biochem Pharma (Mumbai, India), and used without further purification.

General Procedure for Preparation of β-Amino Ketones

One equivalent of $CaCl_2$ was added to an equimolar mixture of acetophenone, benzaldehyde, and N-aryl amine in 5 mL of ethanol and one drop of HCl was added; the resulting mixture was stirred at 60–80° C for 2 h. The obtained solid product was filtered, washed with water, and recrystallized from ethanol. The structures of the compounds were confirmed by spectral analysis (UV, FTIR, and NMR).

RESULTS

Synthesis of β-Amino Ketones

The synthesis of β-amino ketone derivatives was performed according to the method described by Kulkarni et al.[13]; calcium chloride ($CaCl_2$) was used as the catalyst (Fig. 1). The results are summarized in Table 1.

Mannich reactions proceeded smoothly in short time (2 h) in the presence of 1 mol of $CaCl_2$ to give the corresponding products in moderate yield. The maximum yield was obtained with aryl amine (**2e**), but a low yield was obtained with aminothiazole (2 h), 33%.

Spectral Data for the Synthesized β-Amino Carbonyl Compounds (4a–4i)

1,3-Diphenyl-3-(phenylamino)propan-1-one (**4a**). White powder, yield 47.4%, F 170°C, UV_{max} (ACN):260 nm, IR (KBr):3388, 2967, 2869, 1685, 1500, 1405, 869, 711 cm^{-1}. ^1H NMR (300 MHz, *$CDCl_3$)*: 3.21 (dd, 14.8, 9.2Hz, H-2a), 3.63 (dd, 14.8, 8.3, H-2b), 3.79 (dd, 9.2, 8.3, H-3), 8.1 (d,6.7, 2H, H-2', H-6'), 7.45 (dd, 6.7, 6.9, 2H, H-3', H-5'), 7.61 (t, 6.9, 1H, H-4'), 6.67 (d,6.4, 2H, H-2", H-6"), 7.13 (dd, 6.4, 6.7, 2H, H-3", H-5'), 6.81 (t, 6.7, 1H, H-4"), 6.65 (d,7.2, 2H, H-2"', H-6"'), 7.17 (dd, 6.9Hz, 7.2Hz, 2H, H-3"', H-5"'), 6.77 (t, 6.9 Hz, 1H, H-4"'), 4.07(br, NH).

3-[(2-méthylphenyl) amino]-1,3-diphénylpropan-1-one (**4b**). White powder, yield 37.5%, F 156°C, UV_{max} (ACN):302,IR (KBr):3404, 2967, 2869, 1688, 1520, 1454, 869, 711, 629 cm^{-1}. ^1H NMR (300 MHz, *$CDCl_3$)*: 3.2 (dd, 14.8, 9.2, H-2a), 3.65 (dd, 14.8, 8.3, H-2b), 3.77 (dd, 9.2, 8.3, H-3), 8.07 (d,6.7Hz, 2H, H-2', H-6'), 7.44 (dd, 6.7, 6.9Hz, 2H, H-3', H-5'), 7.6 (t, 6.9, 1H, H-4'), 6.65 (d,6.3, 2H, H-2", H-6"), 7.14 (dd, 6.3, 6.5, 2H, H-3", H-5'), 6.79 (t, 6.5, 1H, H-4"), 7.41 (d, 6.9, 1H, H-3"'), 7.25 (dd, 6.9Hz,6.8Hz 1H, H-4"'). 7.38 (dd, 6.8 Hz, 7.1 Hz, 1H, H-5"'),7.19 (d, 7.1 Hz, 1H, H-6"'), 2.44(s,3H, CH3), 4.15(br, 1H, NH).

3-[(3-methylphenyl)amino]-1,3-diphenylpropan-1-one (**4c**). White powder, yield 39.5%, F 160°C, UV_{max} (ACN):275 nm,IR (KBr, cm^{-1}):3420, 1670, 1520, 1416, 876,725 cm^{-1}.^1H NMR (300 MHz, *$CDCl_3$)*: 3.23 (dd, 14.3, 8.9, H-2a), 3.68 (dd, 14.9, 8.3, H-2b), 3.79 (dd, 8.9, 8.3, H-3), 8.13 (d,6.7Hz, 2H, H-2', H-6'), 7.43 (dd, 6.7Hz, 7.01Hz, 2H, H-3', H-5'), 7.59 (t, 7.01, 1H, H-4'), 6.68 (d,6.7Hz, 2H, H-2", H-6"), 7.13 (dd, 6.7, 7.01, 2H, H-3", H-5'), 6.82 (t, 6.7, 1H, H-4"),, 6.58 (dd,2.8, 6.8,1H, H-2"'), 6.71 (d, 6.8,1H, H-4"'), 7.03 (dd, 6.8,6.6, 1H, H-5"'), 6.87 (dd,2.8, 6.8, 1H, H-6"'), 2.47(s, 3H,CH3), 4.17(br, NH).

3-[(3-methoxyphenyl)amino]-1,3-diphenylpropan-1-one (**4d**). White powder, yield 44.5%, F 160°C, UV_{max} (ACN):260 nm,IR (KBr):3404, 2823,2926,1705, 1527,1490,1405,876, 711 cm^{-1}.^1H NMR (300 MHz, *$CDCl_3$)*: 3.28 (dd, 14.7, 8.3, H-2a), 3.68 (dd, 14.7, 8.3, H-2b), 3.82 (dd, 8.9, 8.3, H-3), 7.98 (d,6.8Hz, 2H, H-2', H-6'), 7.47 (dd, 6.8Hz, 7.05Hz, 2H, H-3', H-5'), 7.59 (t, 7.05, 1H, H-4'), 6.66 (d,6.8Hz, 2H, H-2", H-6"), 7.11 (dd, 6.8, 7.05, 2H, H-3", H-5'), 6.82 (t, 6.8, 1H, H-4"), 6.51 (dd,2.7, 6.8,1H,H-2""), 6.67 (d, 6.8, 1H, H-4"'), 7.21 (dd, 6.8,6.6, 1H, H-5"'), 6.77 (dd,2.7, 6.8, 1H, H-6"'), 3.57(s, 3H,O-CH3), 3.99(br, NH).

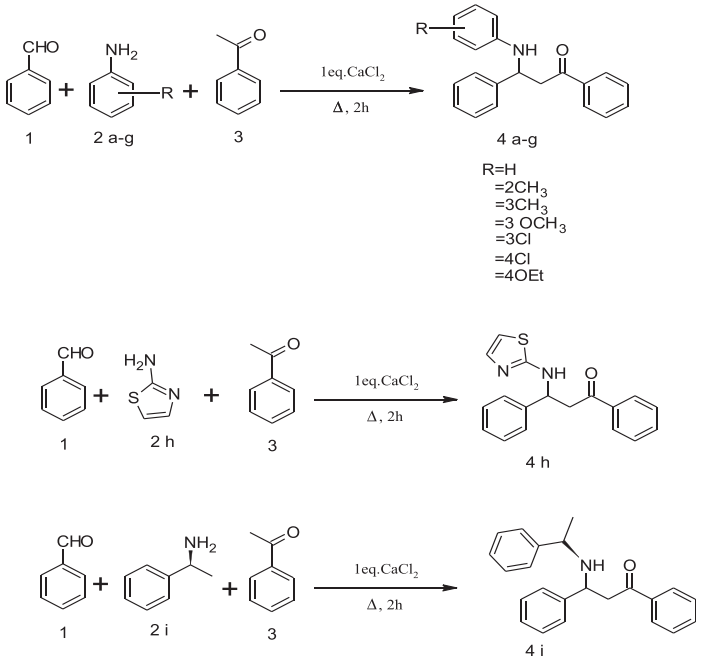
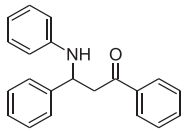
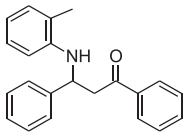
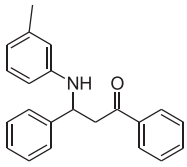
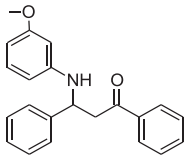
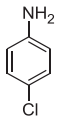
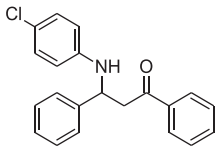
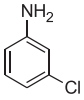
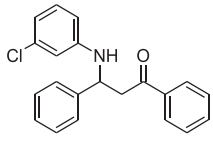


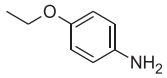
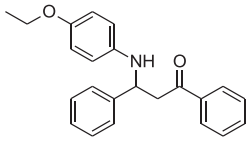
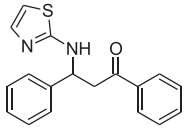
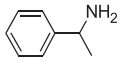
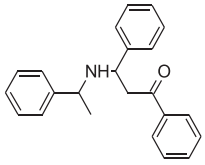
Fig. 1. One-pot Mannich reaction of acetophenone and benzaldehyde and primary amines catalyzed by $CaCl_2$.

TABLE 1. Structures of β-amino ketone derivatives synthesized

Entry	Amine	Structure	Yield[a]/%	M.p./°C
1	 **2a**	 **4a**	**47.4**	**170**
2	 **2b**	 **4b**	**37.46**	**159**
3	 **2c**	 **3c**	**39.5**	**160**
4	 **2d**	 **3d**	**44.43**	**160**
5	 **2e**	 **3**[e]	**70.55**	**168**
6	 **2f**	 **3f**	**38.86**	**168**

(*Continues*)

TABLE 1. (Continued)

Entry	Amine	Structure	Yield[a]/%	M.p./°C
7	 **2g**	 **4g**	**39.53**	**174**
8	 **2h**	 **4h**	**32.73**	**176**
9	 **2i**	 **4i**	**47**	**156**

3-[(4-chlorophényl)amino]-1,3-diphénylpropan-1-one (**4e**). White powder, yield 70.55%, F 168°C, UV_{max} (ACN):260 nm,IR (KBr):3371, 1661, 1500, 1410,875,720 cm^{-1}. ^1H NMR (300 MHz, *$CDCl_3$)*: 3.31 (dd, 14.7, 8.3, H-2a), 3.65 (dd, 14.7, 8.3, H-2b), 3.83 (dd, 8.9, 8.3, H-3), 8.13 (d,6.8Hz, 2H, H-2', H-6'), 7.41 (dd, 6.8Hz, 7.01Hz, 2H, H-3', H-5'), 7.62 (t, 7.05, 1H, H-4'), 6.67 (d,6.8Hz, 2H, H-2", H-6"), 7.18 (dd, 6.8, 7.05, 2H, H-3", H-5'), 6.83 (t, 6.8, 1H, H-4"), 6.59 (d, 8.8, 2H, H-2"', H-6"'), 7.09 (d,8.8,2H,H-3"', H-5"'), 4.11(br, NH).

3-[(3-chlorophényl)amino]-1,3-diphenylpropan-1-one (**4f**). White powder, yield 38.86%, F 168°C, UV_{max} (ACN):260 nm, IR (KBr):3404, 2972,2869, 1694, 1540, 1410, 875, 716 cm^{-1}. ^1H NMR (300 MHz, *$CDCl_3$)*: 3.35 (dd, 13.9, 7.8, H-2a), (dd, 13.9,8.1, H-2b), 3.54 (dd, 7.8,8.1, H-3), 8.07 (d,6.8, 2H, H-2', H-6'), 7.39 (dd, 6.8, 7.01, 2H, H-3', H-5'), 7.61 (t, 7.01, 1H, H-4'),6.85 (d,6.8, 2H, H-2", H-6"), 7.13 (dd, 6.8, 7.01, 2H, H-3", H-5'), 6.86 (t, 6.8, 1H, H-4"), 6.46 (d, 2.7, 1H, H-2"'), 6.68 (d,8.7,1H,H-4"'), 7.01 (dd, 8.7,8.1,1H, H-5"'), 6.59 (dd, 2.7, 8.5, 1H, H-6"'), 4.09(br; NH).

3-[(4-éthoxyphényl)amino]-1,3-diphénylpropan-1-one (**4g**). White powder, yield 39.5%, F 170°C, UV_{max} (ACN):250 nm,IR (KBr, cm^{-1}):3400 (N-H), 3360 (R-CH3), 1689 (C=O),1500(C=C) 1399 (σ CH_2),γ 875 - 711,38. ^1H NMR (300 MHz, *$CDCl_3$)*: 3.37 (dd, 14.7, 8.3, H-2a), 3.71 (dd, 14.7, 8.3, H-2b), 3.83 (dd, 8.9, 8.3, H-3), 8.17 (d,6.8Hz, 2H, H-2', H-6'), 7.49 (dd, 6.8Hz, 7.02Hz, 2H, H-3', H-5'), 7.62 (t, 7.05, 1H, H-4'), 6.68 (d,6.8Hz, 2H, H-2", H-6"), 7.23 (dd, 6.8, 7.05, 2H, H-3", H-5'), 6.87 (t, 6.8, 1H, H-4"), 6.69 (d, 8.8, 2H, H-2"', H-6"'), 7.14 (d,8.8, 2H, H-3"', H-5"'), 3.92(q, 5.6Hz, 2H, O-CH2), 1.97 (t,5.6Hz, 3H, CH3)4.12(br, NH).

1,3-diphenyl-3-(1,3-thiazol-2-ylamino)propan-1-one (**4h**). Yellow powder, yield 32.73%, F 176°C, UV_{max} (ACN):275 nm, IR (KBr):3399, 3175, 1683, 1421, 869, 711cm^{-1}. ^1H NMR (300 MHz, *$CDCl_3$)*: 3.29 (dd, 14.6, 7.6, H-2a), 3.55 (dd, 14.6,7.1, H-2b),3.81 (dd, 7.1,7.6, H-3), 7.92 (d,6.8, 2H, H-2', H-6'),7.44 (dd, 6.8, 6.5, 2H, H-3', H-5'),7.59 (t, 6.5, 1H, H-4'), 6.81 (d,6.3, 2H, H-2", H-6"), 6.67 (dd, 6.3, 6.5, 2H, H-3", H-5'), 7.13 (t, 6.3, 1H, H-4"), 7.15 (d,6.5Hz, 1H, H-3"'), 6.77 (d,6.5Hz,1H,H-4"'), 4.03(br, NH).

1,3-diphenyl-3-[(1-phénylethyl)amino]propan-1-one (**4i**). White powder, yield 47%, F 156°C, UV_{max} (ACN):301 nm, IR(KBR): 3343, 3335, 3024, 2967, 2840, 1679, 1610, 1580, 1495, 1430, 1325, 723, 675 cm^{-1}. ^1H NMR (300 MHz, *$CDCl_3$)*: 3.34 (dd, 14.1, 7.6, H-2a), 3.56 (dd, 14.1,7.2, H-2b), 3.78 (dd, 7.6,7.2, H-3), 8.3 (d,6.7, 2H, H-2', H-6'), (dd, 6.7, 6.5, 2H, H-3', H-5'), (t, 6.5, 1H, H-4'), (d,6.1, 2H, H-2", H-6"), (dd, 6.1, 6.4, 2H, H-3", H-5'), (t, 6.4, 1H, H-4"), 7.18 (d,7.2, 2H, H-2"', H-6"'), 7.37 (dd, 6.9, 7.2, 2H, H-3"', H-5"'), 7.24 (t, 6.9, 1H, H-4"'). 1.52 (d,3.1,3H, CH3), 3.87 (q,3.09, 1H, Ar-CH-N), 4.11(br,NH)

Chiral Separation

Resolution for nine compounds on cellulose derivatives: Chiralcel® OD, Chiralcel® OD-H, Chiralcel® OJ, and

TABLE 2. Chromatographic data for the separation of nine β-amino ketone derivatives on several polysaccharide-based CSPs

CSP	Product	Eluent (% of alcohol)	FR(mL/min)	t_{R1}	t_{R2}	k_1'	k_2'	Rs	α
Chiralcel OD	**4a**	Ethanol 100%	0.5	9.63	10.43	8.63	9.43	1.4	1.09
		ACN 100%	0.5	4.79	4.97	3.79	3.97	0.54	1.04
	4b	ACN 100%	0.5	6.68	7.74	5.8	6.74	1.76	1.18
	4c	Ethanol 100%	0.5	—	—	—	—	—	—
	4d	Ethanol 100%	0.5	—	—	—	—	—	—
	4e	Ethanol 100%	0.5	9.47	—	8.47	—	—	—
		ACN 100%	0.5	7.85	—	6.85	—	—	—
	4f	ACN 100%	0.5	6.38	—	5.38	—	—	—
	4g	ACN 100%	0.5	—	—	—	—	—	—
	4h	ACN 100%	0.5	6.52	—	5.52	—	—	—
	4i	ACN 100%	0.5	—	—	—	—	—	—
Chiralcel OD-H	**4a**	Isopropanol 100%	0.2	21.4	23.84	20.4	22.84	2.04	1.11
		Ethanol 5%	0.5	15.14	16.89	14.14	15.89	2.48	1.12
		ACN 100%	0.5	7.36	7.63	6.36	6.63	0.43	1.04
	4b	ACN 100%	0.5	2.98	3.72	1.98	2.72	1.74	1.37
	4c	Ethanol 5%	0.5	15.43	17.27	14.43	16.27	2.42	1.12
	4d	Ethanol 5%	0.5	15.29	17.06	14.29	16.06	2.42	1.12
	4e	Ethanol 5%	0.5	14.33	15.32	13.33	14.32	1.15	1.07
	4f	Ethanol 5%	0.5	14.34	15.33	13.34	14.33	0.87	1.07
	4g	Ethanol 5%		—	—	—	—	—	—
	4h	Ethanol 5%	0.5	14.3	15.28	13.3	14.28	1.66	1.07
	4i	Ethanol 5%	0.5	—	—	—	—	—	—
	4i	ACN 100%	0.5	—	—	—	—	—	—
Chiralcel OJ	**4a**	Isopropanol 5%	0.5	28.87	30.63	27.87	29.63	1.19	1.06
	4b	Isopropanol 100%	0.3	15.34	23.44	14.34	22.44	5.45	1.56
	4c	Isopropanol (5or100%)		—	—	—	—	—	—
	4d	Isopropanol 100%	0.3	15.47	—	14.47	—	—	—
	4e	Ethanol 5%	0.5	52.55	66.94	51.55	65.94	1.76	1.27
	4f	Isopropanol 100%	0.3	19.27	25.76	18.27	24.76	1.37	1.35
	4g	Isopropanol 100%	0.3	19.27	15.37	18.27	14.37	5.19	0.78
	4g	Ethanol 5%	0.5	24.7	32	23.7	31	2.15	1.3
	4h	Ethanol 5%	0.5	45.1	51.3	44.1	50.3	1.13	1.14
	4h	Isopropanol 5%	0.5	58.4	64.8	57.4	63.8	1.12	1.11
	4i	Isopropanol 5%		—	—	—	—	—	—
Chiralpak AD	**4a**	Isopropanol 100%	0.2	21.2	26.4	20.2	25.4	4.67	1.25
	4b	Isopropanol 100%	0.2	12.16	17.81	11.16	16.81	7.11	1.50
	4c	Isopropanol 100%	0.2	12.1	—	11.1	—	—	—
	4d	Ethanol 100%	0.5	9.518	—	8.518	—	—	—
	4e	Isopropanol 100%	0.2	23.02	27.79	22.02	26.79	2.83	1.21
		Ethanol 5%		36.02	37.02	35.02	36.02	0.29	1.02
	4f	Ethanol 5%	0.5	36.26	37.32	35.26	36.32	0.46	1.3
	4g	Isopropanol 100%	0.2	12.2	20.9	11.2	19.9	7.5	1.77
	4h			—	—	—	—	—	—
	4i			—	—	—	—	—	—
Chiralpak IA	**4a**	Ethanol 20%	0.5	13.45	14.2	12.45	13.2	1.47	1.06
		Ethanol 5%	0.5	23.31	25.84	22.31	24.84	2.89	1.11
	4b			—	—	—	—	—	—
	4c	Ethanol 20%	0.5	—	—	—	—	—	—
	4d			—	—	—	—	—	—
	4e	Ethanol 20%	0.5	15.57	15.94	14.57	14.94	0.41	1.02
	4e	Ethanol 5%	0.5	34.43	36.77	33.43	35.77	1.68	1.06
	4f			—	—	—	—	—	—
	4g			—	—	—	—	—	—
	4h			—	—	—	—	—	—
	4i			10.43	12.5	9.43	11.5	4.86	1.21
Chiralpak IB	**4a**	Ethanol 5%	0.5	19.93	21.19	18.93	20.19	0.93	1.06
		Isopropanol 100%	0.2	19.12	19.82	18.12	18.82	0.62	1.03
	4b	Isopropanol 100%		17.76	19.76	16.76	18.76	0.15	1.11
	4c			18.2	—	17.2	—	—	—
	4d			15.69	—	14.69	—	—	—
	4e	Ethanol 5%	0.5	22.32	23.79	21.32	22.79	1.67	1.06
	4f	Isopropanol 100%	0.2	18.02	19.82	17.02	18.82	0.62	1.10
	4g			—	—	—	—	—	—
	4h	Ethanol 5%	0.5	23.47	25.26	22.47	24.26	0.92	1.07
	4i			—	—	—	—	—	—

Chiralpak® IB, and on amylase derivatives: Chiralpak® IA and Chiralpak® AD are given in Table 2. The mobile phase containing pure alcohols (ethanol or isopropanol) or acetonitrile or mixtures of hexane with ethanol or isopropanol ranging from 90:10 and 90:5 (v/v).

DISCUSSION

Chiralcel® OD-H column shows a higher resolution of the enantiomers of the six compounds**, 4a, 4c, 4d, 4e, 4f, 4h**. The same selector with greater porosity (Chiralcel® OD) shows enantioseparation for only one compound, **4a,** using 100% ethanol as a mobile phase. The immobilized cellulose-based stationary phase in Chiralpak® IB column can separate four compounds, **4a, 4e, 4f,** and **4h**. Cellulose tris-(4-methylbenzoate) chiral selector (Chiralcel® OJ) can also separate four compounds of the series, but with a greater retention time compared to other columns, which exceeds 60 min (**4e** and **4h**).The immobilized amylose-based stationary phase in Chiralpak® IA column can separate the enantiomers of four compounds while its coated type in Chiralpak® AD only shows a higher separation for **4a** and chlorinated derivatives **4e** and **4f** (Fig. 2). Generally, the cellulose derivatives used as chiral selectors are more effective than amylose derivatives. The difference in the capability of chiral discrimination between cellulose and amylose derivatives may be due to the difference in the sizes of the grooves found in the cellulose derivative (Chiralcel® OD-H) as compared to the groove sizes found in the amylose derivative (Chiralpak® AD). It is known that amylose phases have a wider and more helical configuration than the cellulose counterpart.[28]

The resolution of the unsubstituted compound **4a** and the chlorinated derivatives **4e**, **4f** were enantioseparated with a good resolution, *Rs,*>,1.5. However, the resolution of the compounds containing the methyl or methoxy groups in

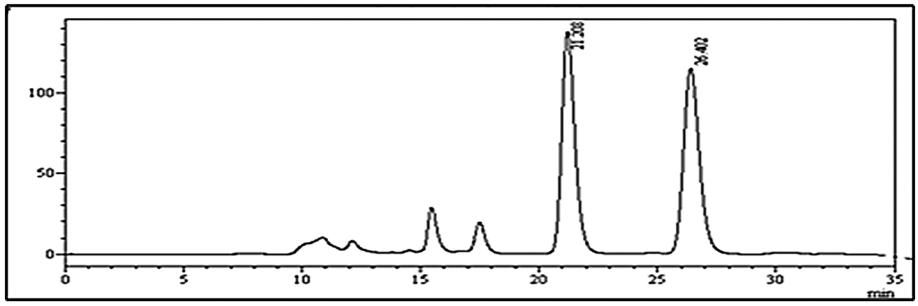


Fig. 2. Chromatogram of compound 4a on Chiralpak® AD, mobile phase 100% isopropanol. The flow rate was 0.3 mL mn^{-1}. α = 1.25, R_S = 4,67.

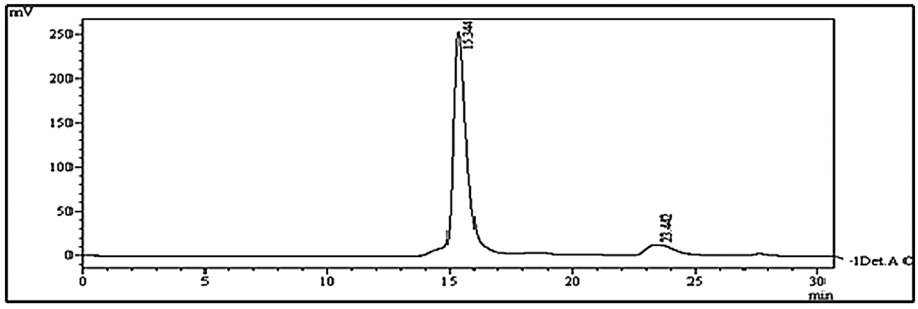


Fig. 3. Chromatogram of compound 4b on Chiralcel® OJ, mobile phase: 100% isopropanol. The flow rate was 0.3 mL mn^{-1}. α = 1.56, R_S = 5.45.

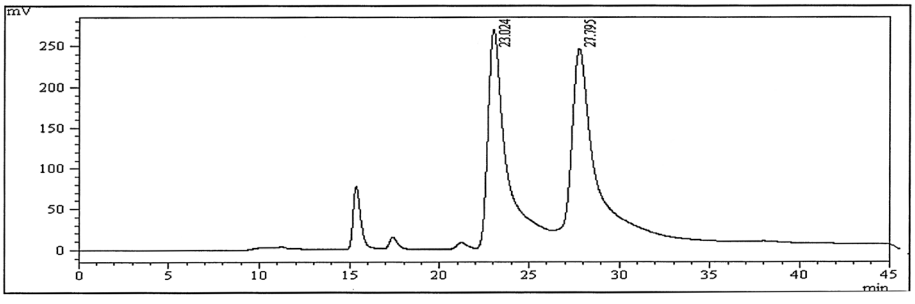


Fig. 4. Chromatogram of compound 4e on Chiralpak® AD, mobile phase: 100% isopropanol. The flow rate was 0.3 mL mn^{-1}. α = 1.21, R_S = 2.83.

meta position of the ring system was achieved only with Chiralcel® OD-H. Furthermore, the resolution of the racemic compound **4b** with methyl group in *ortho* position of ring system was poor (Fig. 3). The enantioseparation of **4f** having an ethoxy group in *para* position was also poor when 100% isopropanol was used as a mobile phase. The enantiomer of compound **4g,** which possesses a thiazole ring in its structure, was separated only on the cellulose derivatives: Chiralcel® OD-H, Chiralcel® OJ, and Chiralpak® IB (Fig. 4). The resolution of compound **4i,** which has two asymmetric centers, was separated only on amylose derivative (Chiralpak® IA). The intermolecular hydrogen bonding, the inductive and electronic effects of the substituents on the aromatic rings and the π–π interactions between the aromatic ring systems in the analytes and the chiral selectors, furthermore, the steric effect contributes to the chiral discrimination between the enantiomers, thus affecting the resolution. This is justified by the separation of the enantiomers of unsubstituted compounds **4a** on all chiral selectors used in this study.

CONCLUSION

The results of this investigation showed that the Chiralcel® OD-H column has higher enantioselectivity than other chiral selectors used in this study since it can resolve about 78% of all compounds tested using mobile phases composed of hexane/ethanol or hexane/isopropanol or isopropanol. The chiral recognition mechanisms that caused the enantioseparation of these racemic β-amino ketones involve intermolecular hydrogen bonding, the inductive and electronic effects of the substituents on the aromatic rings, the π– π stacking interactions between the aromatic rings in the analytes, and the chiral selectors and also the steric effect. All these interactive forces result in chiral discrimination between the enantiomeric pairs.

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