Development of novel chiral capillary electrophoresis methods for the serotonin receptor (5-HT$_{2A}$) antagonist MDL 100,907 (volinanserin) and for its key intermediate compound

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Abstract

Enantioselective capillary electrophoretic methods were elaborated for the determination of the enantiomeric purity of (*R*)-MDL 100,907 and its preparatively resolved key intermediate compound during the synthesis route. The pK_a values of the intermediate compound and the end product determined by CE were 10.5 ± 0.1 and 9.0 ± 0.1, respectively. The enantiopurity of the intermediate compound can be monitored in fully protonated state by applying 15 mM sulfobuthylether-β-cyclodextrin at pH 5 when the peak belonging to the

impurity migrates before the main component. The fact that the consecutive steps of the synthesis do not affect the enantiomeric purity was verified by the other, newly developed CE method. The enantiomers of *rac*-MDL 100,907 were resolved by 15 mM carboxymethyl-γ-cyclodextrin at pH 3. The applicability (selectivity, LOD, LOQ, repeatability, precision and accuracy) of the methods was studied as well.

1. Introduction

MDL 100,907 (Volinanserin; (2,3-dimethoxyphenyl)({1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl})methanol) is a highly selective and potent 5-HT$_{2A}$ receptor antagonist [1]. Its *R*-enantiomer binds to its receptor with higher affinity ($K_i \sim 0.4$ nM) than its racemic form ($K_i \sim 2.1$ nM) [2]. MDL 100,907 has about 100-fold or greater selectivity for 5-HT$_{2A}$ than for the other 5-HT receptor subtypes [3-4]. This drug is widely used in the investigation of the 5-HT$_{2A}$ receptor function [1, 5-8]. The [^{11}C] MDL 100,907, the radioactive derivative is applied in positron emission tomography (PET) for receptor mapping and determination of drug-induced receptor occupancy in pathological conditions (e.g. in schizophreny) [2-6].

The synthesis route (Scheme 1) described by Herth [2] was chosen from the available methods [2, 9-10] to prepare (*R*)-MDL 100,907 for R&D purposes with an 3% enantiomer impurity as maximum. The preparative chiral resolution of the key intermediate (compound **7**: (2,3-dimethoxyphenyl)(piperidin-4-yl)methanol) by the isolation of the diastereomeric salt pairs of the enantiomers of compound **7** prepared with (+)-di-O,O'-*p*-toluyl-D-tartaric acid is an important step. This step is followed by an *N*-alkylation procedure resulting in the (*R*)-MDL 100,907, the end product (compound **10**). The efficiency of the preparative optical resolution of compound **7** can be verified either by NMR [10] or by HPLC [2]. For this

indirect NMR method the sample (compound **8)** goes through a diastereomeric salt derivation with (*R*)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate.

The aim of our study is to develop an easier, direct stereoselective method which consumes less time, solvents and samples. Therefore, novel capillary electrophoretic (CE) methods for the determination of enantiomeric purity of the key intermediate (compound **8**) and the end product (compound **10**) were elaborated.

[Scheme 1]

2. Materials and Methods

2.1. Materials

Anhydrous (+)-di-O,O'-*p*-toluyl-D-tartaric acid were purchased from Sigma-Aldrich (Steinheim, Germany) and all other reagents and solvents, which were used for the synthesis, were supplied by commercial vendors. Background electrolyte (BGE) buffer components phosphoric acid, sodium hydroxide, glacial acetic acid and ethanol were purchased from Merck GmbH (Darmstadt, Germany). Heptakis (2, 3, 6-tri-O-methyl)-β-cyclodextrin (TRIMEB), randomly methylated β-cyclodextrin (RAMEB; Degree of substitution (DS) =12), carboxymethyl-β-cyclodextrin (CMBCD; DS=3.5), carboxymethyl-γ-cyclodextrin (CMGCD; DS=3), sulfobuthylether-β-cyclodextrin (SBEBCD; DS=4) and sulfobuthylether-γ-cyclodextrin (SBEGCD; DS=4) are the products of CycloLab Ltd. (Budapest, Hungary).

2.2. Capillary electrophoresis

Capillary electrophoresis was performed with an Agilent Capillary Electrophoresis [3D]CE system (Agilent Technologies, Waldbronn, Germany) applying bare fused silica capillary of 64.5 cm total and 56 cm effective length with 50 μm I.D. (Agilent Technologies, Santa Clara, CA, USA). On-line UV absorption at 200 nm was monitored by DAD UV-Vis

detector. The capillary was thermostated at 25°C. Between measurements, the capillary was rinsed subsequently with 0.1 M HCl, 1.0 M NaOH, 0.1 M NaOH and distilled water for 3 minutes each and with BGE for 5 minutes. The applied BGEs were 82 mM boric acid (pH 9), 65 mM acetic acid (pH 5), 255 mM acetic acid (pH 4) and 50 mM phosphoric acid (pH 3) and pHs were adjusted by sodium hydroxide. Compounds **7, 8** and **10** (cf. Scheme 1) and *rac*-MDL 100,907 were dissolved in ethanol (1 mg/ml), and they were further diluted with distilled water. Samples were injected by 5×10^3 Pa pressure for 3 sec. Runs were performed in the positive-polarity mode with 30 kV. The resolution (R_s) of enantiomers is given by the following equation [11]:

$$R_s = \frac{1.18 \times (t_2 - t_1)}{w_{(0.5)_1} + w_{(0.5)_2}}$$
Eq. 1

where t is the migration time of the enantiomers (*1, 2* in lower index), $w_{(0.5)}$ is the peak width at half height.

2.3. Estimation of pKa values of the analytes

Experimental pK_a values of the analytes were determined from the effective mobilities (μ_{eff}) measured at a pH where the analytes are in fully ionized state (pH 4) and at pH 9 where the analytes are mixtures of ionized and neutral states. pK_a value of a mono-basic analyte can be calculated from equation 2 [12,13].

$$\mu_{eff} = \frac{\mu_{eff,A^+} \cdot 10^{-pH}}{10^{-pK_a} + 10^{-pH}}$$
Eq. 2

where μ_{eff} is the effective mobility of the analyte, $\mu_{eff,A+}$ is the effective mobilty of the mono-basic analyte in fully ionized state. Predicted thermodynamical pK_a values were calculated using the ChemAxon's MarvinSketch and Marvin Calculator Plugin software (version 5.9.2) called as "Marvin" in the followings.

2.4. Determination of the stability constants of the complexes formed with CDs

The apparent stability constants of complexes (K') were determined at pH 3 and 5. The μ_{eff} was measured without chiral selector and at 5.0, 7.5, 10.0, 12.5, 15.0 mM concentrations of CMBCD, CMGCD, SBEBCD and SBEGCD. The measured mobilities were corrected by the relative viscosity ($\mu'_{eff} = \mu_{eff} \times \eta/\eta_0$) of BGEs containing CDs [14-16] and were plotted against the applied molar concentration of CD derivatives (*[CD]*). Experimental points were fitted with non-linear curve fitting allowing non-limited successive iterations (Origin 8.6 software; goodness of fit: $\chi^2 < 10^{-13}$ and $R^2 > 0.999$) using equation 3 providing the apparent binding constant (K') and the mobilities of the complexes (μ_{compl}) [17-20]:

$$\mu'_{eff} = \frac{\mu_{free} + \mu_{compl} \cdot K' \cdot [CD]}{1 + K' \cdot [CD]} \qquad \text{Eq. 3}$$

where μ_{free} is the mobility of an analyte in BGE without selector. RSD values of the measured and calculated parameters were less than 10%.

2.5. Evaluation of method applicability

The homogeneity of the peaks of the enantiomers of compound **7** and MDL 100,907 was studied by injecting 0.1 mg/ml sample solutions of the raceme compounds. The identification of the peaks was carried out by spiking the raceme sample with the purified *R*-enantiomers (compounds **8** and **10**). Linearity of the methods for the separation of compound **7** or MDL 100,907 was tested by raceme standards at 10, 20, 30, 40, 50, 60, 80, 100 µg/ml concentrations (i.e. 5, 10, 15, 20, 25, 30, 40, 50 µg/ml concentrations for the enantiomers respectively). Intra-day repeatability of the methods was studied by three repetitive injections of 5, 10 and 25 µg/ml concentrations of compounds **7** and *rac*-MDL 100,907, respectively. The inter-day repeatability was determined by repeating the above procedure at three consecutive days. Six-six samples with known concentrations of raceme solutions of compound **7** and MDL 100,907 (5 µg/ml and 10 µg/ml, respectively) were prepared and the

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intra-day accuracies of the methods were established by the determination of the recoveries of the samples calculating their concentrations from the calibration.

3. Results and Discussion

3.1. Method development and optimization

Experimental acidic dissociation constants (pK_a) measured by CE were 10.5 ± 0.1 and 9.0 ± 0.1 for compound **7** and for MDL 100,907, respectively. These data are in a good agreement with the predicted pK_a values (10.05 and 8.75) calculated by "Marvin" software.

Compound **7** and MDL 100,907 have basic character therefore their chiral separations were elaborated at pH 5 and pH 3 in their fully ionized state. According to the literature, the neutral or the anionic CD derivatives should be good choices for selectors when the analytes have cationic character [21]. Two neutral cyclodextrins – TRIMEB and RAMEB - and four acidic CDs - CMBCD, CMGCD, SBEBCD and SBEGCD - were chosen as potential selectors both for compound **7** and *rac*-MDL 100,907. The two neutral CD derivatives can not resolve our analytes. Since the migration times are high and the peak shapes show torsions in the presence of sulfobuthylether CD derivatives at pH 3 these results are not discussed here. Apparent stability constants (K') and the mobilities (μ_{compl}) of negatively charged CD complexes were determined (Table 1) as well. The acidic CD derivatives studied forms more stable complexes with the enantiomers of *rac*-MDL 100,907 than with the enantiomers of compound **7**.

[Table 1]

Although the *K'* values of the enantiomer pairs belonging to a given selector are slightly different these differences still resulted in the predicted migration orders of the enantiomers (EMO) in most but in one cases. Mainly the *K'* of the *S*-enantiomers is higher

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than that of the *R*-enantiomers giving *'RS'* migration order. On the contrary, the reversed *K'* ratio obtained for compound 7 in the presence of SBEBCD is concomitant with the *'SR'* migration order. The corresponding μ_{compl}-s differ from each other substantially only in the case of compound 7 using CMBCD at pH 3 presumably because this CD derivative contains a mixture of isomers [22]. The fact that here $\mu_{compl,R} > \mu_{compl,S}$ could be the reason that the EMO is *'RS'* despite that the EMO predicted from the difference in *K'* should be the opposite of it, namely *'SR'*. The resolutions achieved and the corresponding EMOs are shown in Table 1. In CE good resolutions can be experienced for compound **7** by SBEBCD and SBEGCD at pH 5 and by CMGCD at pH 5 and at pH 3 too. Despite the fact, that improved resolutions can be obtained by CMGCD or SBEGCD as well, SBEBCD results in a more favorable EMO since the impurity migrates before the main component (Fig. 1) in contrast to the reversed EMO obtained by CMGCD or SBEGCD (Table 1). Appropriate separation for the enantiomers of the *rac*-MDL 100,907 can be achieved only by CMGCD at pH 3 (Fig. 1). However, the contaminant *S*-enantiomer migrates slower than the *R*-enantiomer. It is worth mentioning that CMGCD is only partially ionized at pH 3 because its p*Ka* value is approximately 3.75.

[Fig. 1]

The concentrations of the CD derivatives were also optimized according to the measured *K'* and μ_{compl} values. Selectivities slightly increase with increasing concentrations of the selectors in the 5-15 mM concentration range in both cases. According to our experience 15 mM of the selectors provide appropriate resolutions (cf. Fig. 1). Whereas higher concentrations of the selectors may improve selectivity but at the same time undesirable Joule heating and peak torsions can occur. Furthermore, in the presence of higher concentrations of SBEBCD compound **7** co-migrates with EOF and therefore the enantiomer ratio of the analyte can not be evaluated.

The achiral CE methods in the absence of CD selectors at pH 5 and at pH 3 are suitable (R_s = 8.12, α = 1.07 and R_s = 13.36, α = 1.16, respectively) for determining the amount of the remaining compound **7** impurity in MDL 100,907 sample.

3.2. Method application

Applicability of both proposed methods was checked as well [Table 2] according to the recommendations of the Good Laboratory Practice [23]. The values of the intra-day and inter-day repeatability of the *S*-enantiomers were less than 10% being below the acceptable maximum value for this technique. Consequently both methods have good precision. The enantiomer impurities in compound **8** and in the end product, compound **10** were determined as well. The ratios of the contaminant enantiomers are a little bit high but these values are acceptable in the case of a product synthesized for R&D purposes. The preparative optical resolution step of the synthesis needs to be improved if the drug is produced for medical purposes. The enantiomer excess did not increase substantially during the synthesis steps carried out after the optical resolution of the intermediate compound. It means that this last part of synthesis route does not involve steps resulting in racemization.

[Table 2]

4. Conclusions

A chiral CE method was developed using SBEBCD at pH 5 for the evaluation of the enantiopurity of the intermediate compound **8** purified by stereoselective resolution during the synthesis route of (*R*)-MDL 100,907. We determined by the other method elaborated here (applying CMGCD at pH 3) that the consecutive steps of the synthesis could not spoil the enantiomer purity of the end product. This latter method and the method at pH 5 without

selector are equally appropriate to verify the amount of the contaminant intermediate compounds.

References

[1] S.M. Sorensen, J.H. Kehne, G.M. Fadayel, T.M. Humphreys, H.J. Ketteler, C.K. Sullivan, V.L. Taylor, C.J. Schmidt, Characterization of the 5-HT2 receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. J. Pharmacol. Exp. Ther. 266 (1993) 684–691

[2] M.M. Herth, V. Kramer, M. Piel, P.J. Riss, G.M. Knudsen, F. Rösch, Synthesis and in vitro affinities of various MDL 100907 derivatives as potential ^{18}F-radioligands for 5-HT$_{2A}$ receptor imaging with PET. Bioorg. Med. Chem. 17, (2009) 2989-3002.

[3] C. Lundkvist, C. Halldin, N. Ginovart, S. Nyberg, C.-G. Swahn, A. A. Carr, F. Brunner, L. Fardel: [^{11}C]MDL 100907, a radioligand for selective imaging of 5-HT$_{2A}$ receptors with positron emission tomography Life sciences, 58 (1996) 187-192

[4] J. F. López-Giménez, M. T. Vilaró, J. M. Palacios, G. Mengod, [^{3}H]MDL 100,907 labels 5-HT$_{2A}$ serotonin receptors selectively in primate brain, Neuropharmacology 37 (1998) 1147–1158.

[5] T. de Paulis, M-100907 (Aventis). Curr Opin Investig Drugs. 1 (2001) 2123-2132.

[6] H. Watabe, M. A. Channing, M. G. Der, H. R. Adams, E. Jagoda, P. Herscovitch, W. C. Eckelman, R. E. Carson, Kinetic Analysis of the 5-HT$_{2A}$ Ligand [^{ll}C]MDL 100,907 J. Cereb. Blood Flow Metab. 20 (2000) 899-909.

[7] J.-X. Li, C. Crocker, W. Koek, K. C. Rice, C. P. France, Effects of serotonin 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor agonists on schedule-controlled responding in rats: drug combination studies, Psychopharmacology 213 (2011) 489–497.

[8] Shunsuke Nakazawa, Chihiro Yokoyama, Naohiro Nishimura, Tomoko Horisawa, Akihiro Kawasaki, Hiroshi Mizuma, Hisashi Doi, Hirotaka Onoe, Evaluation of dopamine D2/D3 and serotonin 5-HT$_{2A}$ receptor occupancy for a novel antipsychotic, lurasidone, in conscious common marmosets using small-animal positron emission tomography Psychopharmacology 225 (2013) 329–339.

[9] Y.Y. Huang, K. Mahmood,; C. A. Mathis, An efficient synthesis of the precursors of [C-11]MDL 100907 labeled in two specific positions, J. Labelled Compd. Radiopharm. 42 (1999) 949-957.

[10] T. Ullrich, K. C. Rice, A practical synthesis of the serotonin 5-HT2A receptor antagonist MDL 100907, its enantiomer and their 3-phenolic derivatives as precursors for [^{11}C]labeled PET ligands, Bioorg. Med. Chem. 8 (2000) 2427-2432.

[11] S. Fanali, Controlling enantioselectivity in chiral capillary electrophoresis with inclusion-complexation, J. Chromatogr. A 792 (1997) 227–267.

[12] D. Koval, V. Kasicka, J. Jaricek, M. Collinsová, Determination of pKa values of diastereomers of phosphonic pseudopeptides by CZE, Electrophoresis 27 (2006) 4648-4657.

[13] S.K. Poole, S. Patel, K. Dehring, H. Workman, C.F. Poole, Determination of acid dissociation constants by capillary electrophoresis, J. Chromatogr. A 1037 (2004) 445-454.

[14] M.S. Bello, R. Rezzonico, P.G. Rigetti, Capillary electrophoresis instrumentation as a bench-top viscometer, J. Chromatogr. A 569 (1994) 199-204.

[15] V. LemesleLamanche, M. Taverna, D. Wouessidjewe, D. Duchene, D. Ferrier, Determination of the binding constant of salbutamol tonunmodified and ethylated cyclodextrins by affinity capillary electrophoresis J. Chromatogr. A 735 (1996) 321-331

[16] K. Németh, J. R. Mallareddy, C. Domonkos, J. Visy, G. Tóth, A. Péter, Stereoselective analysis of endomorphin diastereomers: Resolution of biologically active analogues by capillary electrophoresis applying cyclodextrins as mobile phase additives, J. Pharm. Biomed.Anal. 70 (2012) 32-39.

[17] S. G. Penn, D. M. Goodall, J. S. Loran, Differential binding of tioconazole enantiomers to hydroxypropyl-beta-cyclodextrin studied by capillary electrophoresis J. Chromatogr. 636 (1993) 149-152.

[18] S. A.C. Wren, Mobility measurements on dansylated amino acids J. Chromatogr. A 768 (1997) 153-159.

[19] A. Salvador, E. Varesio, M. Dreux, J-L. Veuthey, Binding constant dependency of amphetamines with various commercial methylated beta-cyclodextrins, Electrophoresis 20 (1999) 2670-2679.

[20] Y. Tanaka , S. Terabe, Estimation of binding constants by capillary electrophoresis, Journal of Chromatogr.B, 768 (2002) 81–92.

[21] S.A.C. Wren, The separation of enantiomers by capillary electrophoresis Chromatographia 54 (2001) S1-95.

 [22] P. Dubsky, J. Svobodova, B. Gas, Model of CE enantioseparation systems with a mixture of chiral selectors Part I. Theory of migration and interconversion, J. Chromatogr. B, 875 (2008) 30–34.

[23] L. Huber, Good Laboratory Practice for high performance liquid chromatography, capillary electrophoresis and Uv-visible spectroscopy, Hewlett Packard Company, Germany, 1993.

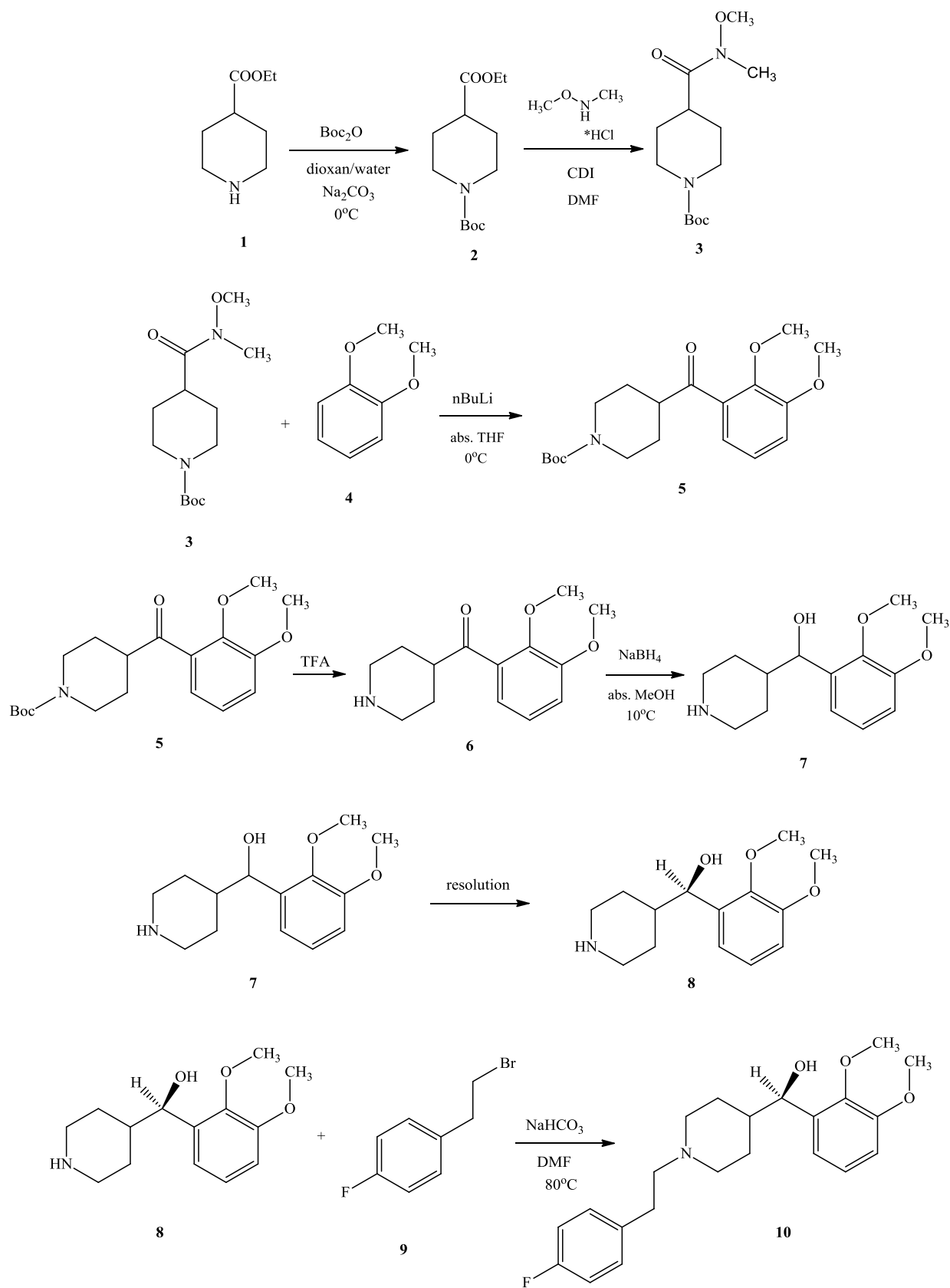# Scheme 1: Synthesis route of (*R*)-MDL 100,907 [2]

Table 1: Resolution (R_s) and migration order of the enantiomers (EMO) using 15 mM selector, stability constants (K') and effective mobilities (μ_{compl}) of the complexes of enantiomers of MDL 100,907 and compound **7** using different CD derivatives at pH 3 and pH 5

| sample | CD | pH | R_s | EMO | K' (M^{-1}) | | | | μ_{compl} (10^{-5} cm^2/Vs) | | | |
			15 mM CD		*S*		*R*		*S*		*R*	
					average	SD	average	SD	average	SD	average	SD
compound **7**	CMBCD	3	0.92	*R,S*	19.0	2.6	25.1	2.5	-1.02	2.47	4.13	1.32
MDL 100,907	CMBCD	3	0.18	*R,S*	109.4	1.4	110.2	2.0	-3.06	0.12	-2.96	0.17
compound **7**	CMGCD	3	3.14	*R,S*	31.1	3.6	28.0	3.0	2.51	1.68	3.12	1.50
MDL 100,907	CMGCD	3	1.84	*R,S*	44.4	1.6	42.0	1.7	-1.63	0.44	-1.66	0.51
compound **7**	CMBCD	5	0.45	*R,S*	75.7	0.9	74.3	0.8	-3.61	0.15	-3.48	0.15
MDL 100,907	CMBCD	5	0		447.5	3.5	447.5	3.5	-14.68	0.05	-14.68	0.05
compound **7**	CMGCD	5	3.48	*R,S*	101.4	2.5	87.4	2.5	-6.69	0.32	-6.08	0.39
MDL 100,907	CMGCD	5	0.80	*R,S*	150.7	3.9	140.4	6.2	-9.69	0.27	-10.05	0.50
compound **7**	SBEBCD	5	1.80	***S,R***	103.0	1.7	106.7	1.2	-5.91	0.20	-6.47	0.14
MDL 100,907	SBEBCD	5	0		428.7	3.1	428.7	3.1	-17.58	0.05	-17.58	0.05
compound **7**	SBEGCD	5	1.80	*R,S*	87.3	0.7	85.0	1.4	-3.89	0.11	-3.21	0.20
MDL 100,907	SBEGCD	5	0.3*	*R,S*	169.6	20.8	161.9	19.7	-8.45	1.14	-8.81	1.18

* Rs using 15 mM SBEGCD
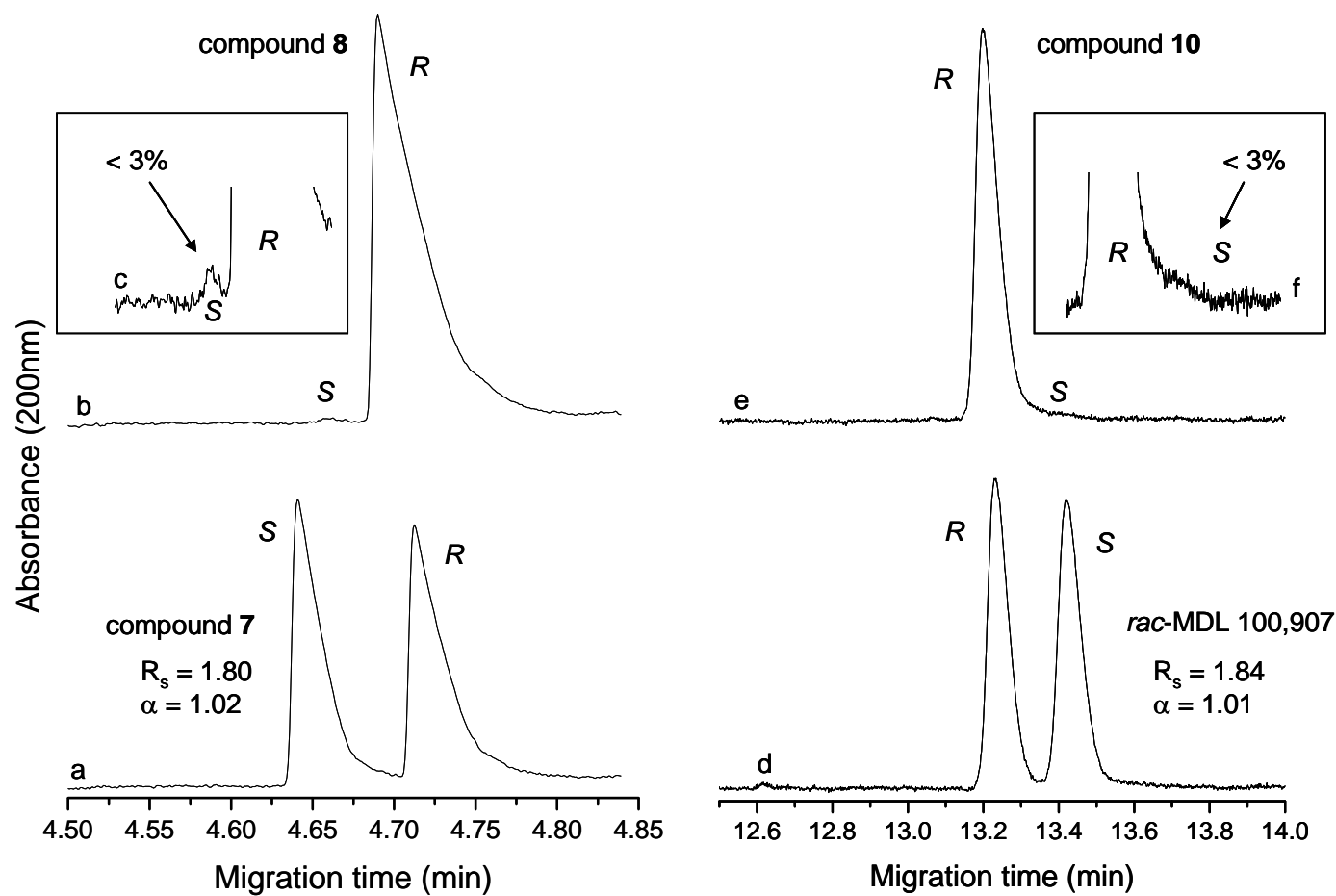
Fig. 1: Stereoselective separation of enantiomers of compound **7** (using 15 mM SBEBCD at pH 5) (a), compound **8** (b); insert (c) represents a region of electropherogram "b" magnified by 50 times. Separation of the enantiomers of *rac*-MDL 100,907 (using 15 mM CMGCD at pH 3) (d), compound **10** (e); insert (f) represents a region of electropherogram "e" magnified by 50 times.

Table 2: Applicability of the two methods developed for the stereoselective analysis of *rac*-MDL 100,907 and of compound **7**

Analytes	compound **7**	MDL 100,907
Conditions	15 mM SBEBCD; pH 5	15 mM CMGCD; pH 3
Enantiomer migration order	*S,R*	*R,S*
LOD (S/N=3)	1.25 µg/ml	2.5 µg/ml
LOQ (S/N=10)	5.0 µg/ml	5.0 µg/ml
Linearity		
concentration range	5-50 µg/ml	5-50 µg/ml
linear equation	y = 0.0625x + 0.8207	y = 0.1336x + 0.0077
coefficient of determination	$r^2 = 0.999$	$r^2 = 0.999$
Precision		
intra-day reproducibility		
RSD (migration time)	0.9 % (1.2; 0.7; 0.9%)	0.9 % (1.0; 0.9; 0.7%)
RSD (area)	5.0 % (7.5; 7.1;0.4%)	2.5 % (3.4; 1.6; 2.5%)
RSD (resolution)	0.6 % (0.9; 0.6; 0.4%)	2.7 % (1.5; 5.0; 1.4%)
inter-day reproducibility		
RSD (migration time)	2.3 % (3.3; 1.6; 2.1%)	0.9 % (1.0; 0.7; 1.1%)
RSD (area)	5.7 % (10; 6.2; 0.9%)	3.4 % (5.6; 2.2; 2.4%)
RSD (resolution)	1.0 % (0.5; 1.4; 1.2%)	2.4 % (3.0; 2.5; 1.8%)
Accuracy		
recovery	93.7 %, 99.5 %, 93.7%, 96.6 %, 93.7 %, 96.6%	97.2 %, 98.7 %, 96.6 %, 97.2 %, 97.2 %, 96.1%
RSD	2.3 %	0.8 %
Bulk		
impurity content	1.3 ±0.8%	2.0± 0.8%

Values refer to the contaminant *S*-enantiomers
y = peak area corrected with migration time, x= concentration of the analytes in µg/ml.
Precision data are presented the average reproducibility values calculated from the individual RSD values in the brackets referring to the 5, 10, 25 µg/ml *S*-enantiomer samples, respectively.
Recovery data were determined at 5 µg/ml and 10 µg/ml concentrations of compound **7** and MDL 100,907, respectively.