Preparation of novel *meta* and *para* substituted *N*-benzyl protected quinuclidine esters and their resolution with butyrylcholinesterase

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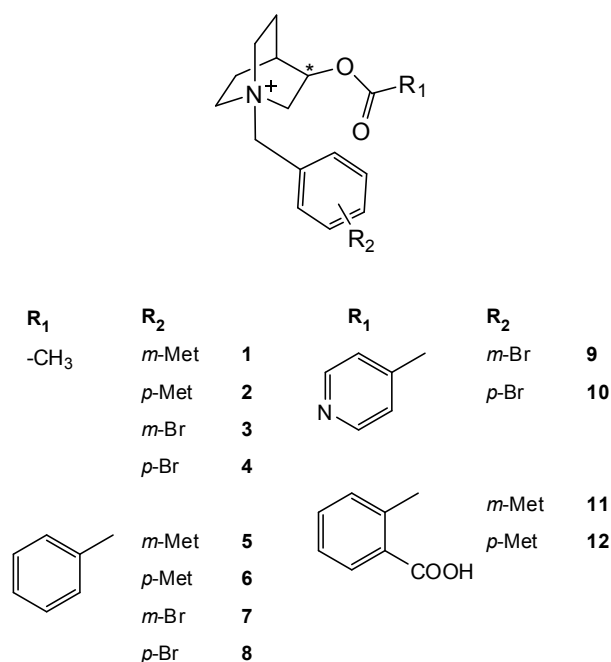
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Abstract: Since the optically active quinuclidin-3-ol is an important intermediate in the preparation of physiologically or pharmacologically active compounds, a new biocatalytic method for the production of chiral quinuclidin-3-ols was examined. Butyrylcholinesterase (BChE; EC 3.1.1.8) was chosen as a biocatalyst in a preparative kinetic resolution of enantiomers. A series of racemic, (*R*)- and (*S*)- esters of quinuclidin-3-ol and acetic, benzoic, phthalic and isonicotinic acids were synthesized as well as their racemic quaternary *N*-benzyl, *meta* and *para N*-bromo and *N*-methylbenzyl derivatives. After the resolution, all *N*-benzyl protected groups were successfully removed by catalytic transfer hydrogenation with ammonium formate (10% Pd-C). Hydrolyses studies with BChE confirmed that (*R*)-enantiomers of the prepared esters are much better substrates for the enzyme than (*S*)-enantiomers. Introduction of bromine atom or methyl group in the *meta* or *para* position of the benzyl moiety resulted in a considerable improvement of the stereoselectivity compared to the non-substituted compounds. Optically pure quinuclidin-3-ols were prepared in high yields and enantiopurity by the usage of various *N*-benzyl protected groups and BChE as a biocatalyst .

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1. Introduction

Butyrylcholinesterase (BChE) is a non-specific ester hydrolyzing enzyme with no endogenous physiological substrate known.[1] It can hydrolyze various esters of choline and other compounds[2] (*e.g.* cocaine, some organophosphorus compounds), and it is important because of several pharmacological and toxicological functions (*e.g.* bioscavenger for the protection of humans against organophosphate toxicity,[3] biomarker of exposure to toxic organophosphorus compounds in food and enviroment[4,5]). This enzyme has not been used to a large extent as biocatalyst in organic chemistry mainly as a result of the affinity toward positively charged substrates.[6] Structural complementarity of choline and quinuclidin-3-ol pointed to racemic quinuclidine derivatives as candidates for kinetic resolution catalyzed by this enzyme.[7-9] Compounds which contain quinuclidin-3-ol moiety are valuable intermediates for the synthesis of optically active pharmaceuticals,[10] thus, efficient synthesis of chiral quinuclidin-3-ols is necessary for the preparation of biologically active compounds. In our previous work, various chiral quaternary esters of quinuclidin-3-ol were subjected to hydrolysis in the presence of BChE,[11] and it was shown that hydrolysis is highly stereoselective. Kinetic studies revealed the preference of BChE toward (*R*)-enantiomers of quinuclidin-3-ol compounds. Therefore, we decided to synthesized a series of new quaternary esters with *meta*- and *para*-bromo and *meta*- and *para*-methyl benzyl protecting groups at the quinuclidine nitrogen atom, to determine whether the substituent at the benzyl moiety can further enhance enantioselectivity of the hydrolysis. A series of racemic, (*R*)- and (*S*)- esters of quinuclidin-3-ol and acetic, benzoic, phthalic and isonicotinic acids were synthesized as well as their racemic quaternary *meta* and *para* *N*-bromo and *N*-methylbenzyl derivatives, Figure 1. After the kinetic resolution using BChE as a catalyst, all *N*-benzyl protected groups were successfully removed by catalytic transfer hydrogenation with ammonium formate (10% Pd-C), Scheme 1. The stereoselectivity of hydrolysis with horse serum BChE was investigated. A method for the preparation of chiral (*R*)-esters and (*S*)-quinuclidin-3-ol by the enzymic kinetic resolution on a preparative scale was examined.

Figure 1. Synthesized quinuclidinium esters

R$_1$	R$_2$		R$_1$	R$_2$	
-CH$_3$	*m*-Met	1		*m*-Br	9
	p-Met	2		*p*-Br	10
	m-Br	3			
	p-Br	4			
	m-Met	5		*m*-Met	11
	p-Met	6		*p*-Met	12
	m-Br	7			
	p-Br	8			

2. Results and Discussion

Twelve quaternary racemic esters of quinuclidin-3-ol and acetic, benzoic, isonicotinic and phthalic acids were synthesized, and a method for their resolution with butyrylcholinesterase was explored, Scheme 1. All esters were synthesized by the esterification reaction of quinuclidin-3-ol with appropriate anhydride in a good yields. Quaternization of esters with substituted *meta* and *para*- bromo and methyl benzyl bromides followed. The purity and structure of all compounds were determined by IR, MS, one- and two-dimensional ^{1}H and ^{13}C NMR. Quaternary racemic esters **1–12** were then hydrolyzed using horse serum BChE as a catalyst. The hydrolysis was stopped when 50% of the ester was hydrolyzed, Table 1. Acetates, benzoates. isonicotates and phthalates were hydrolyzed with different rates. Change of the acyl moiety of the substrate effected activity of the enzyme: the fastest reactions were those of acetates and isonicotinates, while benzoates and phthalates were hydrolyzed much slower. The best substrate for the enzyme was isonicotinate **10**, while benzoate **8** was the worst substrate. All reactions proceeded slower than the reactions of the appropriate tested non-substituted *N*-benzyl derivatives indicated that *meta* and *para* substituent at the *N*-benzyl protecting group generally lowered the affinity of the enzyme toward the substrates.
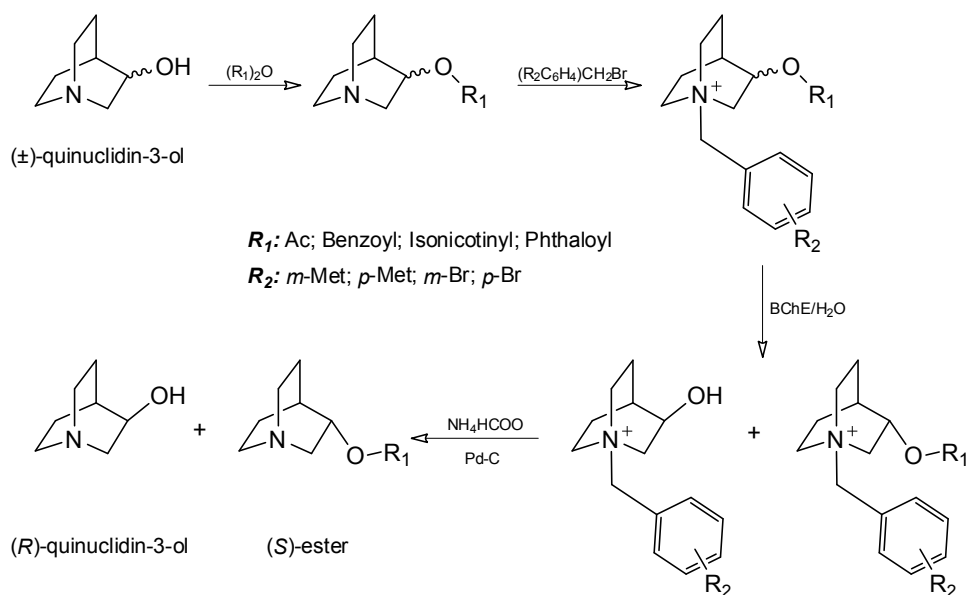
Scheme 1.

R₁: Ac; Benzoyl; Isonicotinyl; Phthaloyl

R₂: *m*-Met; *p*-Met; *m*-Br; *p*-Br

Table 1. Hydrolysis of compound **1-12** catalyzed with butirylcholinesterase: optical purities of products were determined after the catalytic transfer hydrogenation.

Compound	Time (BChE hydrolysis) /min	$[\alpha]_D^{26}$ (*S*)-ester	$[\alpha]_D^{26}$ (*R*)-quinuclidin-3-ol	Optical purity (*S*)-ester /%	Optical purity (*R*)- quinuclidin-3-ol /%
1	80	-140	- 80	88	89
2	60	- 140	- 90	88	100
3	60	-140	-80	88	89
4	60	-140	-80	88	89
5	185	- 82.3	- 60	82	67
6	258	- 100	- 60	100	67
7	360	-62	-70	62	78
8	1320	-100	-70	100	78
9	60	-200	-70	95	78
10	45	-200	-70	95	78
11	170	110	-70	85	78
12	75	100	-80	77	89

After the kinetic resolution, the obtained mixtures of quaternary *N*-benzyl esters and 3-hydroxyquinuclidinium derivatives were subjected to the catalytic transfer hydrogenation, and chiral (*S*)-quinuclidine esters and (*R*)-quinuclidin-3-ol were isolated. Specific optical rotation values were determined with an Optical Activity LTD automatic polarimeter and are presented in Table 1. Enantiopurity of the obtained esters or quinuclidin-3-ol for compounds

2, **6** and **8**–**10** were higher than 95% while the optical purity for other compounds varied from 62–89%. This data imply that BChE is adequate biocatalyst for the kinetic resolution of *N*-benzyl esters: by varying the reaction time, both enantiomers of quinuclidi-3-ol can be obtained in very high optical purity. The enantiopurest (*S*)-esters were benzoates **6** and **8**, compounds with the *para*-bromo and *para*-methyl *N*-benzyl protecting groups. At the same time, the reaction stereoselectivity of derivatives **5** and **7** with *meta* substituents at the benzyl moiety were much lower.

To identify the binding interactions of the enzyme with the benzoate esters, molecular docking study was performed using Autodock 4.0.2. The flexible ligands **5**–**8** were docked to the active site of BChE by using the default settings of Lamarckian genetic algorithm. A general trend in binding of all benzoates was observed: due to the bulkiness of the molecules, productive binding of (*R*)- and (*S*)-esters are not the one with the lowest energies, Figure 2.

Figure 2. Substrate-BChE complexes derived from docking studies **(a) 5-*R*** (carbon atoms dark grey) and **6-*R*** (carbon atoms light grey); **(b) 7-*R*** (carbon atoms dark grey) and **8-*R*** (carbon atoms light grey); **(c) 5-*S*** (carbon atoms dark grey) and **6-*S*** (carbon atoms light grey); **(d) 7-*S*** (carbon atoms dark grey) and **8-*S*** (carbon atoms light grey). BChE active site is represented by three structurally important amino acids. Hydrogen atoms are omitted for clarity with the exception of the polar ones.
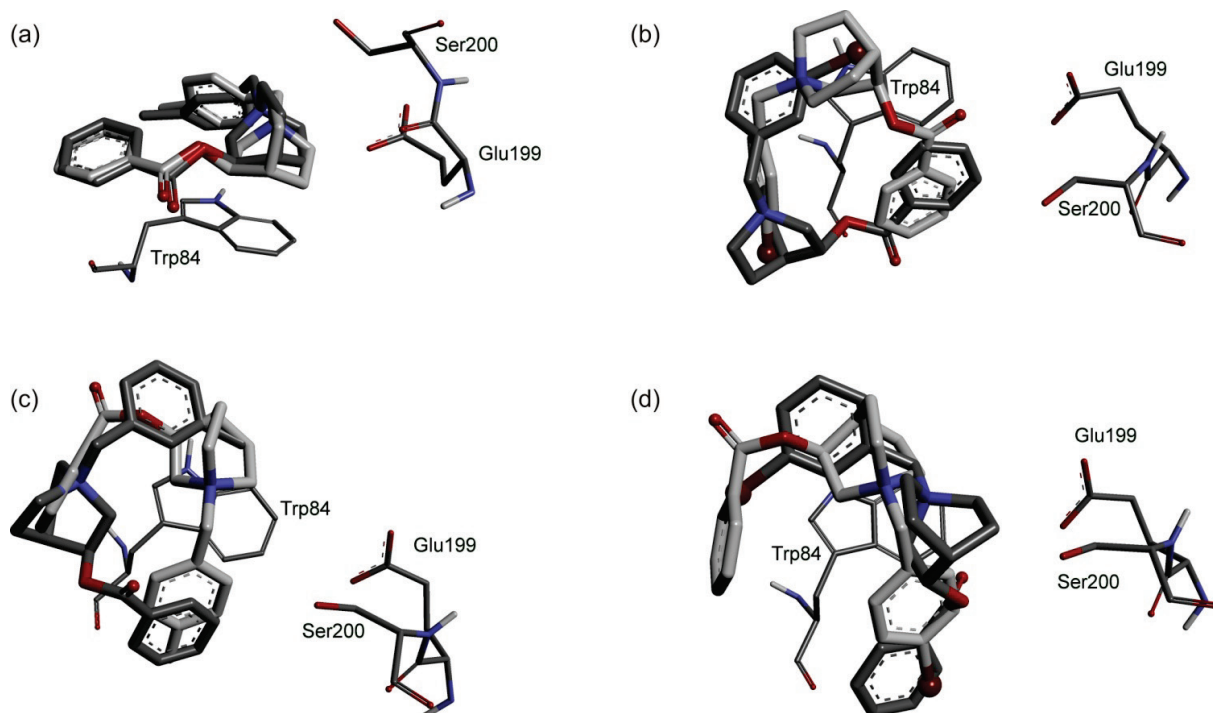
That can provide an explanation why the rate of hydrolysis is slower compared to *N*-methyl and non-substituted *N*-benzyl derivatives.[11] Thus, both enantiomers of compounds have to rotate inside the active site for hydrolyses to occur. There is a difference in accommodation of bromo and methyl groups as a result of their different size and electron features. Comparison of *meta* and *para* derivatives reveals that position of the substituent determines the modes of binding of substrates: only in the case of **5-*R*** and **6-*R*** (Figure 2. (a)) the position of the methyl substituent does not influence significantly the geometry of the substrate in the most stable complex, while in all other complexes there is a radical change in the position of carbonyl group as well as two aromatic moieties.

3. Experimental Section

All reagents and solvents were analytical grade or purified with standard procedure described in literature. All melting points were determined on a Melting Point B-540 apparatus (Büchi, Germany) and are uncorrected. IR spectra were recorded with a Perkin-Elmer FTIR 1725 X spectrometer. ^{1}H and ^{13}C 1D and 2D NMR spectra were recorded with Varian XL-GEM 600 spectrometer at room temperature. Chemical shifts are given in ppm downfield from TMS as internal standard. The reactions with enzyme were carried out in Heidolph UNIMAX 1100 Shaker. BChE (EC 3.1.1.8), type IV-S lyophilized powder from horse serum (Sigma Chemical Co.) was used without further purification. Optical rotations were measured on an Optical Activity AA-10 automatic polarimeter at ambient temperature. Acetate, benzoate, phthalate and isonicotinate esters were synthesized as described previously.[12] Chiral (*R*)- and (*S*)-quinuclidin-3-ols were obtained by the resolution of racemic quinuclidin-3-yl acetates with D-and L-tartaric acid.[13] Standards of chiral alcohol and esters were prepared: (*R*)-quinuclidin-3-ol: $[\alpha]_D^{26}$ -90° (c=0,02, 1M HCl); (*S*)-Quinuclidin-3-yl acetate: $[\alpha]_D^{26}$ -160° (c=0,3, CHCl$_3$); (*S*)-Quinuclidin-3-yl benzoate: $[\alpha]_D^{26}$ -100° (c=0,34, EtOH); (*S*)-Quinuclidin-3-yl isonicotinoate: $[\alpha]_D^{26}$ -211° (c=0,3, CHCl$_3$); (*S*)-Quinuclidin-3-yl phthalate: $[\alpha]_D^{26}$ 130° (c=1,0, EtOH);

3.1. General procedure for quaternization of esters

The appropriate ester (1.5 mmol) was dissolved in dry acetone. Substituted *N*-benzyl bromide (1.7 mmol) was slowly added to the solution. The mixture was left at room temperature and after 24 h the crystals were formed. They were washed several times with dry

ether and dried under reduced pressure to give the title compounds as white crystals (unless stated otherwise).

3-Acetoyloxy-1-(3-methylbenzyl)quinuclidinium bromide (**1**). Yield: 41%; m.p. 196.3-197.8°C; IR (cm^{-1}): 2969 (C-H), 2890 (C-H), 1275 (C=O), 1254 (C-O), 1042 (C-N); ^{1}H-NMR δ: 1.89 – 2.05 (m, 2 H, H$_5$), 2.07 (s, 3H, C$\underline{H}_3$C=O), 2.15-2.23 (m, 2H, H$_8$), 2.32-2.35 (s, 3H, CH$_3$Bz), 2.38-2.40 (m, 1H, H$_4$), 3.62-3.78 (m, 1H, H$_7$), 3.82-3.89 (m, 2H, H$_6$), 3.93-4.04 (m, 1H, H$_{2b}$), 4.24-4.32 (dd, 1H, H$_{2a}$), 5.00-5.10 (m, 3H, H$_3$ and CH$_2$Bz), 7.23-7.44 (m, 4 H, H$_2$H$_4$H$_5$H$_6$Bz); ^{13}C-NMR δ: 18.39 (C$_5$), 21.28 (C$_4$), 21.29 (CH$_3$Bz), 21,37 (C$_8$), 24.69 (C$\underline{H}_3$C=O), 52.87 (C$_6$), 53.69 (C$_7$), 59.90 (C$_2$), 66.70 (CH$_2$Bz), 67.16 (C$_3$), 126.63 (C$_1$Bz), 129.06 (C$_5$Bz), 130.45 (C$_6$Bz), 131.33 (C$_2$Bz), 133.75 (C$_4$Bz), 139.13 (C$_3$Bz), 170.02 (C=O). ESMS**:** *m/z* (calcd for C$_{17}$H$_{24}$NO$_2^+$ 274.18) found 274.2.

3-Acetoyloxy-1-(4-methylbenzyl)quinuclidinium bromide (**2**). Yield: 63%; m.p 174.9-175.6°C; IR (cm^{-1}) : 2945 (C-H), 1720 (C=O), 1241 (C-O), 1025 (C-N); ^{1}H-NMR δ: 1.89 – 2.00 (m, 2 H, H$_5$), 2.08 (s, 3H, C$\underline{H}_3$C=O), 2.14-2.19 (m, 2H, H$_8$), 2.33 (s, 3H, CH$_3$Bz), 2.35-2.39 (m, 1H, H$_4$), 3.62-3.67 (m, 2H, H$_7$), 3.83-3.86 (m, 2H, H$_6$), 3.93-3.95 (m, 1H, H$_{2b}$), 4.22-4.29 (m, 1H, H$_{2a}$), 4.97-5.07 (m, 3H, H$_3$ and CH$_2$Bz), 7.15-7.18 (m, 2H, H$_3$H$_5$Bz), 7.37-7.44 (m, 2H, H$_2$H$_6$Bz); ^{13}C-NMR δ: 17.78 (C$_5$), 20.47 (C$_4$), 20.76 (CH$_3$Bz), 21.37 (C$_8$), 24.23 (C$\underline{H}_3$C=O), 52.50 (C$_6$), 53.14 (C$_7$), 59.36 (C$_2$), 65.95 (CH$_2$Bz), 66.69 (C$_3$), 123.25 (C$_1$Bz), 129.25 (C$_3$C$_5$Bz), 132.71 (C$_2$C$_6$Bz), 140.13 (C$_4$Bz), 169.76 (C=O). ESMS**:** *m/z* (calcd for C$_{17}$H$_{24}$NO$_2^+$ 274.18) found 274.2.

3-Acetoyloxy-1-(3-bromobenzyl)quinuclidinium bromide (**3**). Yield: 63%; m.p. 194-196°C; IR (cm^{-1}): 3050, 2955, 2870, 1720, 1430, 1371, 1244, 1026; ^{1}H-NMR δ: 1.92-2.03 (m, 2H, H$_5$), 2.04-2.09 (s, 3H, C$\underline{H}_3$C=O), 2.18-2.20 (m, 2H, H$_8$), 2.39-2.40 (m, 1H, H$_4$), 3.70-3.93 (m, 5H, H$_6$, H$_7$ and H$_{2b}$), 4.33-4.34 (dd, 1H, H$_{2a}$), 5.08-5.21 (m, 3H, H$_3$ and CH$_2$Bz), 7.36-7.41 (m, 4H, H$_2$H$_4$ H$_5$H$_6$Bz); ^{13}C NMR δ: 17.89 (C$_5$), 20.51 (C$_4$), 20.90 (C$_8$), 24.15 (C$\underline{H}_3$C=O), 52.89 (C$_6$), 53.34 (C$_7$), 59.56 (C$_2$), 64.75 (CH$_2$Bz), 66.57 (C$_3$), 122.58 (C$_1$Bz), 128.70 (C$_3$Bz), 130.30 (C$_6$Bz), 131.80 (C$_5$Bz), 133.25 (C$_4$Bz), 135.25 (C$_2$Bz), 169.67 (C=O). ESMS**:** *m/z* (calcd for C$_{16}$H$_{21}$BrNO$_2^+$ 338.08) found 338.2.

3-Acetoyloxy-1-(4-bromobenzyl)quinuclidinium bromide (**4**). Yield: 60%; m.p. 239-241°C; IR (cm^{-1}): 3050, 2956, 2870 (v$_s$ C-H); 1735, 1488,1422, 1363, 1239, 1029; ^{1}H-NMR δ: 1.89-2.02 (m, 2H, H$_5$), 2.04-2.10 (s, 3H, C$\underline{H}_3$C=O), 2.17-2.21 (m, 2H, H$_8$), 2.38-2.39 (m, 1H, H$_4$), 3.64-3.72 (m, 2H, H$_7$), 3.77-3.95 (m, 3H, H$_6$ and H$_{2b}$), 4.28-4.29 (dd, 1H, H$_{2a}$), 5.06-5.07 (m, 3H, H$_3$), 5.14-5.21 (m, 2H, CH$_2$Bz), 7.50-7.52 (m, 2H, H$_2$H$_6$ Bz), 7.56-7.58 (m, 2H, H$_3$H$_5$Bz); ^{13}C

NMR δ: 17.87 (C_5), 20.47 (C_4), 20.88 (C_8); 24.15 ($\underline{C}H_3C=O$), 52.80 (C_6), 53.21 (C_7), 59.51 (C_2), 64.79 (CH_2Bz), 66.57 (C_3); 124.91(C_1Bz), 125.36 (C_4Bzl), 131.96 (C_3C_5Bz), 134.47 (C_2C_6Bz), 169.65 (C=O). ESMS: *m/z* (calcd for $C_{16}H_{21}BrNO_2^+$ 338.08) found 338.2.

3-Benzoyloxy-1-(3-methylbenzyl)quinuclidinium bromide (**5**). Yield: 89%, m.p. 145.8-146.9°C; IR (cm^{-1}): 2969 (C-H), 1710 (C=O), 1453 (C=C), 1278 (C-O), 1024 (C-N); ^{1}H-NMR δ: 1.95-2.29 (m, 4H, H_5 and H_8), 2.32 (s, 3H, CH_3Bz), 2.54-2.57 (m, 1H, H_4), 3.76-4.13 (m, 5H, H_6, H_7 and H_{2b}), 4.37-4.47 (m, 1H, H_{2a}), 5.10 (s, 2H, CH_2Bz), 5.33-5.36 (m, 1H, H_3), 7.19-7.29 (m, 2H, H_2H_6Bn), 7.39-7.46 (m, 2H, H_2H_6Bz), 7.53-7.59 (m, 3H, $H_3H_4H_5Bn$), 8.01-8.04 (m, 2H, H_4H_5Bz); ^{13}C NMR δ: 18. 54 (C_5), 21.28 (CH_3Bz), 21.37 (C_8), 24.94 (C_4), 53.31 (C_6), 53.98 (C_7), 59.97 (C_2), 66.81 (CH_2Bz), 67.79 (C_3), 126.69 (C_1Bz), 128.81 (C_3Bz), 129.08 (C_3C_5Bn), 128.38 (C_{10}), 129.86 (C_2C_6Bz), 130.43 (C_2C_6Bn), 130.43 (C_5Bz), 131.29 (C_4Bz), 133.82 (C_4Bn), 139.31 (C_3Bz), 165.40 (C=O). ESMS: *m/z* (calcd for $C_{22}H_{26}NO_2^+$ 336.20) found 336.2.

3-Benzoyloxy-1-(4-methylbenzyl)quinuclidinium bromide (**6**). Yield : 81%, m.p. 209.6-210.4°C; IR (cm^{-1}): 2968 (C-H), 2879 (C-H), 1721 (C=O), 1449 (C=C), 1271 (C-O), 1022 (C-N); ^{1}H-NMR δ: 1.95-2.28 (m, 4H, H_5 and H_8), 2.56 (s, 3H, CH_3Ph), 2.52-2.61 (m, 1H, H_4), 3.68-4.16 (m, 5H, H_6, H_7 and H_2), 4.32-4.41 (m, 1H, H_2), 5.11 (s, 2H, H_{16}), 5.32-5.35 (m, 1H, H_3), 7.18-7.23 (m, 2H, $H_{11 \text{ and }}H_{15}$), 7.43-7.48 (m, 2H, $H_{18 \text{ and }}H_{22}$), 7.51-7.62 (m, 3H, H_{12}, H_{13} and H_{14}), 8.01-8.04 (m, 2H, H_{19} and H_{21}); ^{13}C NMR δ: 18. 55 (C_5), 21.30 (C_{23}), 21.38 (C_8), 24.98 (C_4), 53.35 (C_6), 53.93 (C_7), 59.99 (C_2), 66.68 (C_{16}), 67.77 (C_3), 123.64 (C_{20}), 128.61($C_{12}C_{14}$), 128.76 (C_{17}), 129.87 ($C_{18}C_{22}$), 129.94 ($C_{11}C_{15}$), 133.22 ($C_{19}C_{21}$), 133.73 (C_{13}), 140.92 (C_{10}), 165.51 (C_9). ESMS: *m/z* (calcd for $C_{22}H_{26}NO_2^+$ 336.20) found 336.2.

3-Benzoyloxy-1-(3-bromobenzyl)quinuclidinium bromide (**7**). Yield: 78%; m.p. 140-142°C; IR (cm^{-1}): 3050, 2966, 2885, 1716, 1450,1409, 1214, 1012; ^{1}H-NMR δ: 1.94-2.27 (m, 4H, H_5 and H_8), 2.53-2.55 (m, 1H, H_4), 3.76-4.09 (m, 5H, H_6, $H_{7 \text{ and }}H_{2b}$), 4.37-4.45 (dd, 1H, H_{2a}), 5.10 (s, 2H, CH_2Bz), 5.33-5.36 (q, 1H, H_3), 7.24-7.29 (m, 1H, H_4Bn), 7.38-7.78 (m, 6H, $H_2H_3H_5H_6Bn$ and H_5H_6Bz), 7.99-8.01 (d, 2H, H_2H_4Bz); ^{13}C NMR δ: 19.95 (C_5), 20.10 (C_8), 24.90 (C_4), 53.07 (C_6), 53.58 (C_7), 55.21 (C_2), 65.68 (CH_2Bzl), 67.68 (C_3), 123.12 (C_1Bz), 128.60 (C_1Bn), 129.07 (C_3C_5Bn), 129.09 (C_3Bz), 129.89 (C_2C_6Bn), 130.82 (C_4Bn), 132.30 (C_6Bz), 133.70 (C_5Bz), 133.81 (C_4Bz), 135.72 (C_2Bz), 165.56 (C=O). ESMS: *m/z* (calcd for $C_{21}H_{23}BrNO_2^+$ 400.09) found 400.2.

3-Benzoyloxy-1-(4-bromobenzyl)quinuclidinium bromide (**8**). Yield: 67%; m.p. 84-86°C; IR (cm^{-1}): 3050, 2963, 2885, 1716, 1409, 1214, 1012; ^{1}H-NMR δ: 2.03-2.27 (m, 4H, H_5 and H_8), 2.55-2.56 (m, 1H, H_4), 3.72-4.05 (m, 5H, H_6, $H_{7 \text{ and }}H_{2b}$), 4.35-4.45 (dd, 1H, H_{2a}), 5.18 (s, 2H,

CH$_2$Bz), 5.33-5.36 (q, 1H, H$_3$), 7.27-7.29 (m, 1H, H$_4$Bn), 7.41-7.59 (m, 6H, H$_2$H$_3$H$_5$H$_6$Bn and H$_2$H$_6$Bz), 7.99-8.01 (d, 2H, H$_3$H$_5$Bz); ^{13}C NMR δ: 19.39 (C$_5$), 20.10 (C$_8$), 24.89 (C$_4$), 53.07 (C$_6$), 53.74 (C$_7$), 57.53 (C$_2$), 62.16 (CH$_2$Bz), 67.73 (C$_3$), 125.43 (C$_1$Bz), 125.61 (C$_1$Bn), 128.62 (C$_3$C$_5$Bn), 128.70 (C$_4$Bz), 128.73 (C$_2$C$_6$Bz), 129.87 (C$_2$C$_6$Bn), 131.51 (C$_4$Bn), 132.51 (C$_3$C$_5$Bz), 165.56 (C=O). ESMS: *m/z* (calcd for C$_{21}$H$_{23}$BrNO$_2$$^+$ 400.09) found 400.2.

3-Isonicotinoyloxy-1-(3-bromobenzyl)quinuclidinium bromide (**9**). Yield: 78%; m.p. 251-255°C; IR (cm^{-1}): 3050, 2963, 2870, 1720, 1468,1406, 1217, 1039; ^{1}H-NMR δ: 1.94-1.95 (m, 2H, H$_5$), 2.10-2.12 (m, 2H, H$_8$), 2.27-2.31 (m, 1H, H$_4$), 3.48 – 4.19 (m, 5H, H$_7$, H$_6$ and H$_{2b}$), 4.28-4.30 (dd, 1H, H$_{2a}$), 4.48-4.51 (m, 2H, CH$_2$Bz), 5.29-5.30 (q, 1H, H$_3$), 7.33 (t, 1H, H$_5$Bz), 7.56-7.70 (m, 2H, H$_4$H$_6$Bz), 7.77-7.78 (m, 1H, H$_2$Bz), 7.89-7.90 (m, 2H, H$_2$H$_6$Py), 8.76-8.77 (m, 2H, H$_3$H$_5$Py); ^{13}C NMR δ: 17.99 (C$_5$), 20.82 (C$_8$), 24.32 (C$_4$), 52.51 (C$_6$), 53.52(C$_7$), 59.40 (C$_2$), 64.77 (CH$_2$Bz); 68.10 (C$_3$), 122.52 (C$_2$C$_6$Py), 122.73 (C$_1$Py), 128.52 (C$_1$Bz), 130.35 (C$_3$C$_5$Py), 130.78 (C$_6$Bz), 131.33 (C$_5$Bz), 133.21 (C$_3$Bz), 133.38 (C$_2$Bz), 135.23 (C$_4$Bz), 135.54 (C=O). ESMS: *m/z* (calcd for C$_{20}$H$_{22}$BrN$_2$O$_2$$^+$ 401.09) found 401.1.

3-Isonicotinoyloxy-1-(4-bromobenzyl)quinuclidinium bromide (**10**). Yield: 78%; m.p. 119-121°C; IR (cm^{-1}): 3050, 2953, 2870, 1731, 1462,1409, 1214, 1012; ^{1}H-NMR δ: 1.92-2.33 (m, 4H, H$_5$ and H$_8$), 2.57-2.58 (m, 1H, H$_4$), 3.50-4.24 (m, 5H, H$_6$, H$_7$ $_{and}$ H$_{2b}$), 4.32-4.51 (dd, 1H, H$_{2a}$), 5.25 (s, 2H, CH$_2$Bz), 5.29-5.41 (q, 1H, H$_3$), 7.47-7.66 (m, 4H, H$_2$H$_3$H$_4$H$_5$Bz), 7.89-7.91 (m, 2H, H$_2$H$_6$Py), 8.78-8.80 (m, 2H, H$_3$H$_5$Py); ^{13}C NMR δ: 18.47 (C$_5$), 21.30 (C$_8$), 24.05 (C$_4$), 53.06 (C$_6$), 53.61 (C$_7$), 59.78 (C$_2$), 65.33 (CH$_2$Bz), 68.33 (C$_3$), 119.46 (C$_2$C$_6$Py), 125.69 (C$_1$Py), 131.95 (C$_2$C$_6$Bz), 134.49 (C$_3$C$_5$Bz), 136.03 (C$_1$Bz); 144.13 (C$_4$Bz), 151.03 (C$_3$C$_5$Py), 164.38 (C=O). ESMS: *m/z* (calcd for C$_{20}$H$_{22}$BrN$_2$O$_2$$^+$ 401.09) found 401.1.

3-Phthaloyloxy-1-(3-methylbenzyl)quinuclidinium bromide (**11**). Yield : 49%, yellow viscous oil; IR (cm^{-1}): 3344 (O-H), 2923 (C-H), 1721 (C=O), 1488 (C=C), 1284 (C-O), 1039 (C-N); ^{1}H-NMR δ: 0.88-0.99 (m, 2H, H$_5$), 1.20-1.48 (m, 2H, H$_8$), 1.80-2.15 (m, 1H, H$_4$), 2.32-2.40 (CH$_3$Bz), 3.38-3.97 (m, 5H, H$_6$, H$_7$ and H$_{2b}$), 4.65-4.71(m, 1H, H$_{2a}$), 5.17-5.24 (s, 2H, CH$_2$Bz), 5.29-5.36 (s, 1H, H$_3$), 7.08-7.31 (m, 4H, H$_3$H$_4$H$_5$H$_6$Pht), 7.56-8.25 (m, 4H, H$_2$H$_4$H$_5$H$_6$Bz); ^{13}C-NMR δ: 13.36 (CH$_3$Bz), 18.05 (C$_5$), 20.62 (C$_8$), 21.23 (C$_4$), 26.22 (C$_6$), 29.98 (C$_7$), 64.05 (C$_3$), 67.53 (CH$_2$Bz), 67.83 (C$_2$), 125.49 (C$_2$Bz), 128.41-129.29 (C$_2$C$_4$C$_5$C$_6$Bz), 130.55 (C$_1$Pht), 130.89-131.97 (C$_3$C$_4$C$_5$C$_6$Pht), 132.75 (C$_1$Bz), 135.17 (C$_2$Pht), 138.35 (C$_3$Bz), 168.09 (COOH), 171.03 (C=O). ESMS: *m/z* (calcd for C$_{23}$H$_{26}$NO$_4$$^+$ 380.19) found 380.3.

3-Phthaloyloxy-1-(4-methylbenzyl)quinuclidinium bromide (**12**). Yield: 81%, yellow viscous oil; IR (cm^{-1}): 3419 (O-H), 2957 (C-H), 1722 (C=O), 1458 (C=C), 1285 (C-O), 1039 (C-N); ^{1}H-NMR δ: 1.77-1.87 (m, 4H, H_5 and H_8), 2.27-2.35 (m, 1H, H_4), 2.28 (s, 3H, CH_3Bz), 3.45-3.52 (m, 5H, H_6, H_7 and H_{2b}), 3.94-3.90 (m, 1H, H_{2a}), 4.84 (s, 2H, CH_2Bz), 4.51 (q, 1H, H_3); 7.11-7.16 (m, 4H, $H_2H_3H_5H_6Bz$), 7.27-7.36 (m, 2H, H_4H_5Pht), 7.45-7-52 (m, 2H, H_3H_6Pht), 8.19-8.24 (m, 1H, COOH); ^{13}C-NMR δ: 17.97 (C_5), 21.23 (C_4), 21.57 (C_8), 26.92 (CH_3Bz), 53.27 (C_7), 54.36 (C_6), 63.77 (C_2), 64.09 (C_3), 67.63 (CH_2Bz), 123.44 (C_1Bz), 129.93 (C_3C_6Pht), 131.04 ($C_2C_3C_5C_6Bz$), 132.43 (C_4Pht), 132.88 (C_5Pht), 134.11 (C_1C_2Pht), 140.89 (C_4Bz), 170.95 (C=O, COOH). ESMS: *m/z* (calcd for $C_{23}H_{26}NO_4^+$ 380.19) found 380.3.

3.2. Kinetic resolution with BChE

The appropriate quaternary ester (40 mg) was dissolved in a minimal volume of 0.1 M phosphate buffer. The solution was placed in shaker for 10 minutes and 100 μL BChE (10 mg/ml) was added to a reaction mixture. Reaction was stopped when 50% of the ester was hydrolyzed by adding a 10 ml of ethanol. The reaction mixture was dried under the reduced pressure and extracted with chloroform. The extract was dried over Na_2SO_4 and evaporated under reduced pressure.

3.3. Catalytic transfer hydrogenation

A dry methanol (3 ml) was added to the residue. Methanol solution was placed into a 2-bottom flask and 10% Pd-C was added (40 mg). Ammonium formate (30 mg) was added in a single portion. The reaction mixture was stirred at the reflux temperature. After the completion of the reaction, it was filtered through a Celite pad, and washed with 5 ml of dry methanol. The filtrate was dried under the reduced pressure, made alkaline with saturated aqueous solution of K_2CO_3 and extracted with chloroform. The extract was dried and evaporated under reduced pressure. Products were separated by column chromatography (aluminum oxide 90 active neutral (70-230 mesh ASTM), (Merck, Darmstadt, Germany) with chloroform-methanol solution (9:1) as eluent.

4. Conclusions

A series of novel *meta* and *para* substituted *N*-benzyl protected quinuclidinium esters were prepared and stereoselectivity of hydrolysis catalyzed with horse serum BChE was investigated. The kinetic resolutions were performed at 37 °C in 0.1 M phosphate buffer, pH 7.4. The hydrolyses of (*R*)-esters proceeded much faster than that of (*S*)-enantiomers. Introduction of *N*-benzyl *para* and *meta* bromine atom or methyl group resulted in a significant improvement of the stereoselectivity compared to non-substituted *N*-benzyl protected group. Thus, optically pure quinuclidin-3-ol derivatives were prepared in high yields and enantiopurity by the esterification, quaternization with derivatives of benzyl bromide, BChE-catalysed hydrolysis and finally catalytic transfer hydrogenation.

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