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Synthesis of conformationally restricted acetylcholine analogues. Comparing lipase-mediated resolution with simulated moving bed chromatography of arylated β-hydroxy-pyrrolidines

Ricardo de L. Barreto,[a] Marcos J. S. Carpes,[a] César C. Santana[b] and Carlos R. D. Correia[a,*]

[a]*Chemistry Institute, State University of Campinas—UNICAMP, C.P. 6154, CEP 13084-971, Campinas, São Paulo, Brazil*
[b]*School of Chemical Engineering, State University of Campinas—UNICAMP, C.P. 6066, CEP 13093-970, Campinas, São Paulo, Brazil*

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Abstract—The enantiodivergent synthesis of new, conformationally restricted acetylcholine analogues was accomplished using arylated endocyclic enecarbamates as key intermediates. Stereoselective hydroboration of the aryl enecarbamates provided the corresponding aryl-β-hydroxy-pyrrolidines. Acetylation, deprotection, followed by N-bismethylation, led to the desired betaine products. The kinetic resolution of the intermediate alcohols was performed by lipase-mediated hydrolysis of its acetate derivatives, resulting in excellent enantioselectivities ($E > 100$). An enzymatic enantiopreference predicted by the Kazlauskas's model was confirmed following Riguera's protocol. Finally, chromatographic resolution of racemic alcohol **5a** was evaluated by semi-preparative scale chiral simulated moving bed chromatography and its performance compared with biocatalysis.
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1. Introduction

Acetylcholine (AcCh, Fig. 1) is an important neurotransmitter acting at the central nervous system and the neuromuscular junction. Its deficiency in the cholinergic system[1] is thought to be one of the causes of Alzheimer's disease (AD). AD is a severe neurodegenerative disorder leading to a characteristic deterioration of intellectual capacity, executive functions and personality changes.[2]

Alzheimer's disease is the most common cause of dementia in the elderly and experts estimate that 22 million people around the world will be afflicted with AD by 2025.[3] In the United States, the annual direct and indirect costs involved in caring for people with AD approached 100 billion dollars in 1991.[4] One of the current therapeutic treatments consists of the use of acetylcholinesterase (AcChE) inhibitors,[5] which increase the efficiency of cholinergic transmission by preventing the hydrolysis of released AcCh, thus raising its level at the cholinergic synapse.

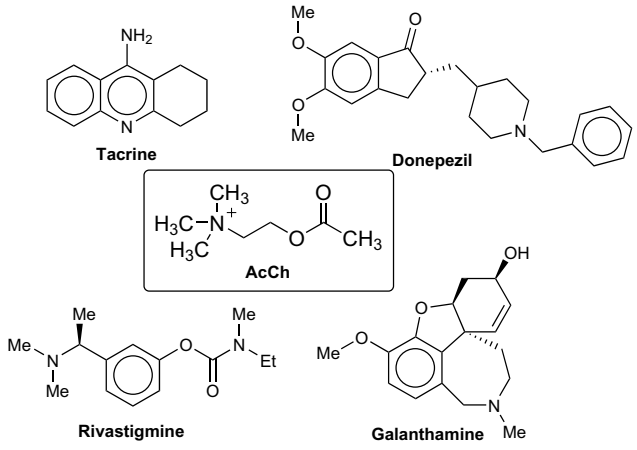

Figure 1. Some examples of AcChE inhibitors.

The cholinergic concept has led to the development and commercialization of several drugs (Fig. 1) for the

* Corresponding author. Tel.: +55 19 3788 3114; fax: +55 19 3788 3023; e-mail: roque@iqm.unicamp.br

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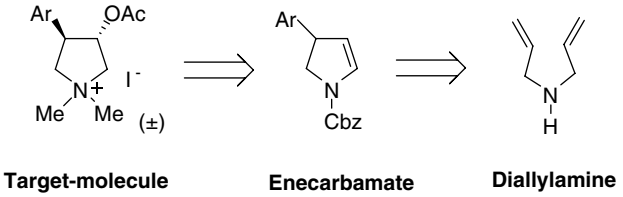
symptomatic treatment of AD: tacrine (*Cognex*®, Warner–Lambert), donepezil (*Aricept*®, Eisai/Pfizer), rivastigmine (*Exelon*®, Novartis) and galanthamine (*Reminyl*®, Shire/Johnson & Johnson).

Based on these considerations, we envisaged the synthesis of new conformationally restricted AcCh analogues as potential AcChE inhibitors. The synthetic route involved an arylated endocyclic enecarbamate as a key intermediate, which can be obtained in a few steps from commercial diallylamine, followed by the Heck arylation of 3-pyrroline, a protocol previously developed in our research group[6] (Scheme 1).



Scheme 1. The synthetic strategy.

Due to the increasing interest of the pharmaceutical industries in single enantiomers of chiral drugs,[7] there has also been special interest in the association of biocatalysis and simulated moving bed (SMB) technology to organic synthesis as an alternative method to asymmetric synthesis.

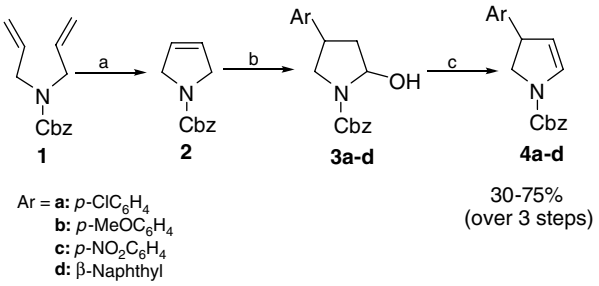
Over the last two decades, biocatalytic systems have gained growing importance in organic synthesis for the preparation of chiral compounds.[7,8] A great number of enzymes are commercially available for a variety of biotransformations. In particular, lipases[9] (triacylglycerol hydrolases, E.C. 3.1.1.3) are often used for the kinetic resolution of racemates.

The SMB chromatography is a large-scale version of traditional high-performance liquid chromatography (HPLC), but, unlike normal HPLC, it operates continuously in an automated multicolumn chromatographic system, with reduced solvent consumption and purification costs.[10] This process consists of simulating the countercurrent movement of adsorbent, producing two outlet streams, one rich in the more adsorbable component (extract stream) and the other rich in the less adsorbable one (raffinate stream), which is particularly appropriate for binary separations such as racemates.[11]

Herein we report the synthesis of new conformationally restricted AcCh analogues and compare the resolution power of a lipase with that of the SMB in the separation of alcohol intermediates employed in the synthesis of the new AcCh analogues.

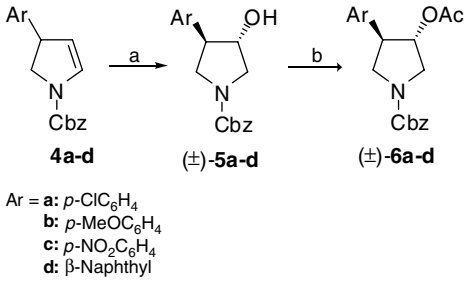
2. Results and discussion

The synthesis features a ring-closing metathesis of the *N*-Cbz-protected diallylamine **1**, followed by the Heck arylation of *N*-Cbz-3-pyrroline **2** with arenediazonium tetrafluoroborates, and dehydration of the intermediate lactamol **3** to give the arylated endocyclic enecarbamates **4a–d** (Scheme 2).



Scheme 2. Reagents and conditions: (a) [Ru], CH_2Cl_2, rt, 5 h; (b) $Pd(OAc)_2$, ArN_2BF_4, CH_3CN/H_2O, rt, 3 h; (c) $(CF_3CO)_2O$, 2,6-lutidine, 0 °C to rt, 2 h, reflux, 30 min.

Enecarbamates **4a–d** were then subjected to hydroboration–oxidation, providing exclusively (±)-(*trans*)-alcohols **5a–d**. As described in the literature,[12] hydroboration of substituted enecarbamates occurs in the β position and in a stereoselective manner, resulting in the preferential attack of the borane on the less esterically hindered side of the double bond. Acetylation of alcohols **5a–d** resulted in the racemic acetate derivatives (±)-*trans*-**6a–d** (Scheme 3).

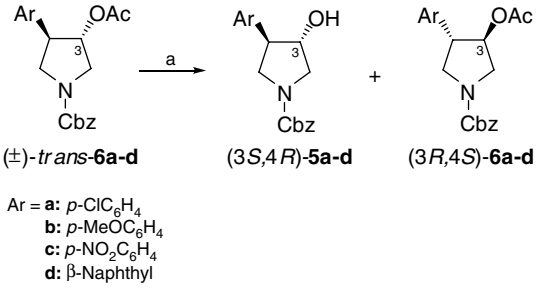


Scheme 3. Reagents and conditions: (a) (1) $BH_3{\cdot}SMe_2$, 0 °C to rt, 3 h, (2) H_2O_2, NaOH, 0 °C (45 min), rt (45 min), 90%; (b) $(CH_3CO)_2O$, DMAP, Pyr. CH_2Cl_2, rt, 2 h, 95%.

Acetate derivatives (±)-*trans*-**6a–d** were then submitted to enzymatic hydrolysis (Scheme 4) using lipase *Pseudomonas* cepacia[13] (lipase PS from *Amano Pharmaceutical* Co.) to furnish alcohols (3*S*,4*R*)-*trans*-**5a–d** and acetates (3*R*,4*S*)-*trans*-**6a–d** with excellent enantioselectivities (Table 1). The enzymatic enantiopreference was predicted to be (*S*) by the Kaslauskas's empiric model.[14]

In most experiments, enantioselectivities were excellent ($E > 100$), especially for the alcohol bearing the large β-naphthyl substituent (entry 4), in accordance with the Kazlauskas's model. Conversions (C) below 50% (entry 1) lead to considerable losses in the ee_s values, while C values slightly above 50% raise ee_s and diminishes ee_p values (entry 4).

In order to confirm the foreseen enzymatic enantiopreference employing Kazlauskas's model, we determined the



Scheme 4. Reagents and conditions: (a) Lipase PS-AK, phosphate buffer (10% toluene), pH 7.0.

Table 1. Enzymatic hydrolysis of acetate derivatives **6a–d**[a]

Entry	Ar	ee_p[b]	ee_s[c]	C[d]	E[e]
1	*p*-ClC_6H_4	98.8	58.0	37.0	288
2	*p*-$MeOC_6H_4$	96.3	97.1	50.2	228
3	*p*-$NO_2C_6H_4$	92.9	93.0	50.0	94
4	β-Naphthyl	97.2	99.4	50.6	386

[a] Quantitative treatment of enzymatic kinetic resolutions developed by Sih et al.[15]
[b] Enantiomeric excess (%) of the product.
[c] Enantiomeric excess (%) of the substrate.
[d] Extent of conversion (C), where $C = ee_s/(ee_s + ee_p)$.
[e] Enantiomeric ratio (E), where $E = \ln[(1-c)\cdot(1-ee_s)]/\ln[(1-c)\cdot(1+ee_s)]$.

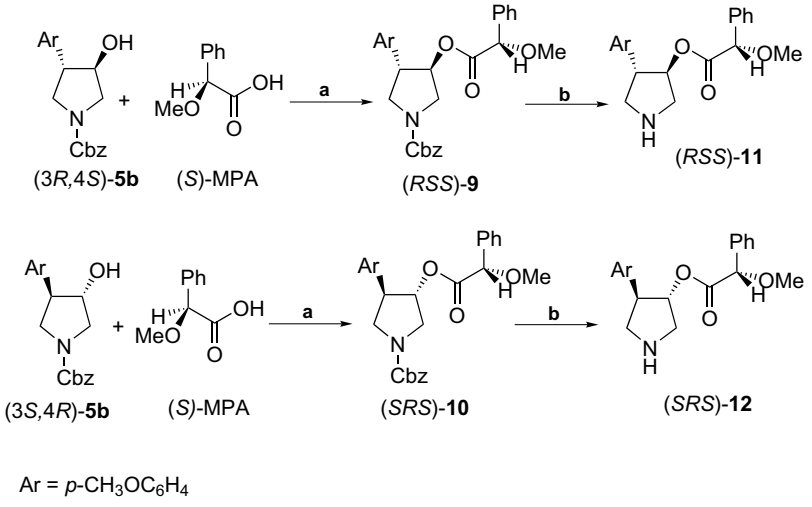
absolute configuration of the resolved alcohol **5b** (*p*-MeO–Ph) using Riguera's protocol,[16] after reacting it with (*S*)-methoxyphenylacetic acid (MPA). The ^1H NMR spectra of the corresponding diastereomeric esters (*RSS*)-**9** and (*SRS*)-**10** were rather complex, probably because of the presence of rotamers. Therefore, we then removed the Cbz protecting groups and compared the spectra of free amines (*RSS*)-**11** and (*SRS*)-**12** (Scheme 5).

Evaluating the differences in chemical shifts ($\Delta\delta$) between the signals for hydrogens H-2 (L_1 neighbourhood), H-4 and H-5 (L_2 neighbourhood) from the corresponding diastereomeric esters (*RSS*)-**11** and (*SRS*)-**12**, it was possible to calculate $\Delta\delta^{RS}$ values of +0.30 for L_1 and −0.26 for L_2 (Scheme 6), which is in good agreement with the (*S*) enantiopreference as predicted by the Kazlaukas's model.

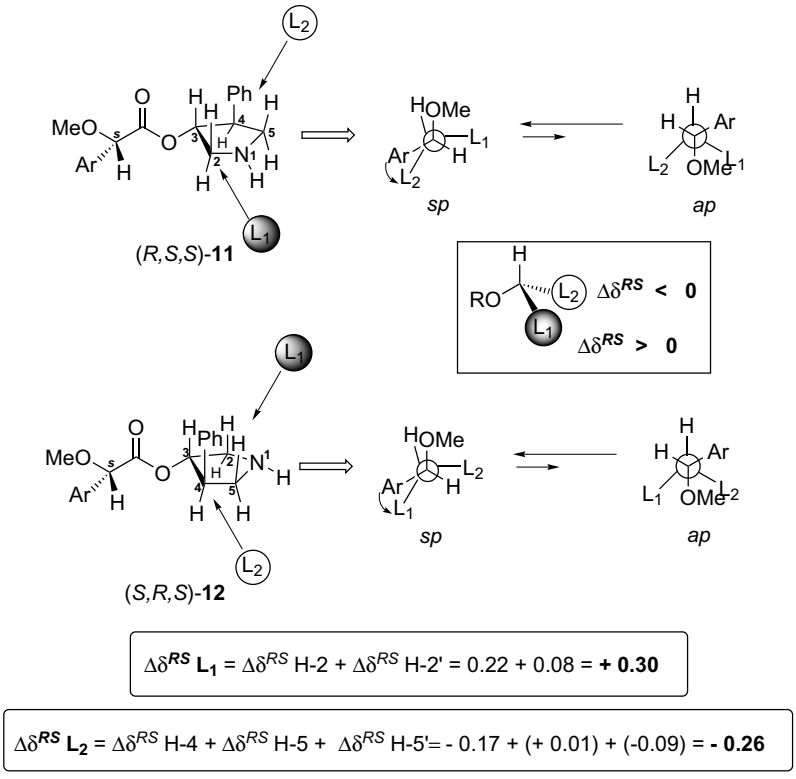
We also compared the resolution performance of the lipase with the SMB technology. For this purpose we ran a chromatographic resolution of racemic alcohol **5a** (Ar = *p*-Cl–C_6H_4) in a semi-preparative SMB equipment composed of eight columns having cellulose tris(3,5-dimethylphenylcarbamate) as the chiral stationary phase. The SMB run was performed with a mixture of hexane and isopropyl alcohol (85:15, v/v) as eluent, a feed concentration of 2 g/L, and a flow rate of 0.1 mL/min. Under these operational conditions, we obtained the raffinate stream with the enantiomerically enriched alcohol (*R*)-(−)-**5a** in 96.9% ee and $[\alpha]_D^{20} = -19.0$ (*c* 3.0, CH_2Cl_2). On the other hand, alcohol (*S*)-(+)-**5a** was obtained in the extract stream in only 83.5% ee and $[\alpha]_D^{20} = +15.0$ (*c* 3.0, CH_2Cl_2).[17]

Comparing the results of the chiral SMB and biocatalysis, in terms of productivity (grams of material processed per day), for this particular molecule, SMB was a more effective methodology for the resolution of the enantiomers. While the enzyme resolution required 30 days to process 0.5 g of racemic alcohol, the laboratory-scale SMB unit was able to process the same amount in 2 days. Nevertheless, it is important to point out that there was a decrease in enantioselectivity for the (*S*)-enantiomer (98.8–83.5% ee) but a considerable increase for the (*R*)-enantiomer (58.0–96.9% ee).

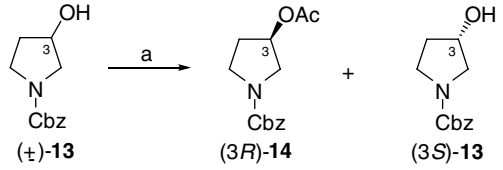
Despite the advantages of SMB chromatography, we found that some separations can be excessively laborious in terms of optimizing chromatographic parameters. This was the case for the simpler alcohol (±)-*N*-Cbz-3-hydroxy-pyrrolidine **13**. Various separation conditions were tested in order to obtain acceptable resolutions (changes in eluent polarity, flow rates, use of additives and different chiral stationary phases), but all attempts were fruitless. On the other hand, enzymatic resolution of pyrrolidine **13**, via



Scheme 5. Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, 75%; (b) $Pd(OH)_2/C$, H_2, MeOH, rt, 2 h, 40%.



Scheme 6. Conformational models of the diastereomeric esters.

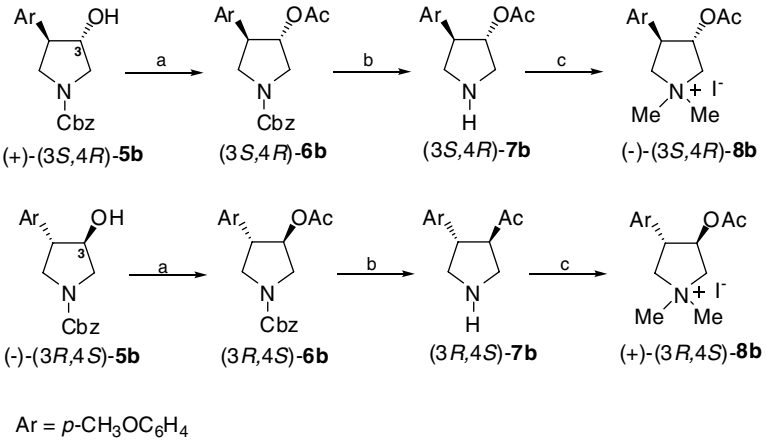


Scheme 7. Reagents and conditions: (a) lipase PS in sol gel AK, vinyl acetate, CH_2Cl_2, 10 days.

transesterification in organic solvent[18] (Scheme 7), was very effective in terms of enantioselectivity, but still time demanding (10 days to achieve 45% conversion). The resolved acetylated alcohol (3*R*)-**14** was obtained in >99% ee and the remaining alcohol (3*S*)-**13** in 81.3% ee.

The absolute configuration was attributed by comparing the specific rotation obtained for acetate (3*R*)-**14** $[\alpha]_D^{20} = -13.5$ (*c* 1.48, CH_3OH) with that reported in the literature.[19] The Kazlauskas's model was not applicable in this case due to the similar sizes of the substituents.

Having enough quantity of the resolved alcohols (+)-(3*S*,4*R*)-**5b** ($[\alpha]_D^{20} = +6.4$ (*c* 1.6, CH_2Cl_2)) and (−)-(3*R*,4*S*)-**5b** ($[\alpha]_D^{20} = -6.7$ (*c* 1.2, CH_2Cl_2)), we then synthesized



Scheme 8. Reagents and conditions: (a) $(CH_3CO)_2O$, DMAP, Pyr., CH_2Cl_2, rt, 2 h, 95%; (b) $Pd(OH)_2/C$, H_2, MeOH, rt, 2 h, 88%; (c) MeI (excess), $NaHCO_3$, CH_2Cl_2, reflux, 4 days, 100%.

the desired quaternary ammonium salts (−)-(3*S*,4*R*)-**8b** ($[\alpha]_D^{20} = -13.5$ (*c* 1.3, H_2O)) and (+)-(3*R*,4*S*)-**8b** ($[\alpha]_D^{20} = +17.5$ (*c* 2.0, H_2O)), by the sequence shown in Scheme 8 encompassing acetylation, removal of the Cbz protecting group and bis methylation.

3. Conclusion

In conclusion, new and conformationally constrained acetylcholine derivatives were synthesized in an enantioselective manner in nine steps from diallylamine in overall yields of ~15%. The key step in the synthesis involved a kinetic resolution performed by lipase PS or by chiral simulated moving bed chromatography. A comparison of the two technologies was performed using key intermediate **5a**. The absolute stereochemistry of the alcohol intermediates was determined using the procedure of Riguera's. Evaluation of the acetylcholinesterase inhibition capability of these new acetylcholine derivatives are in progress.

4. Experimental

4.1. General

Reagents and solvents are of commercial grade and were used as supplied, except when specified in the experimental procedure. In cases where dry solvents were employed, they were distilled from calcium hydride or Na. ^{1}H NMR and ^{13}C NMR data were recorded on a Varian Gemini 2000 (7.0 T) or Varian Inova (11.7 T) spectrometer with tetramethylsilane as internal standard (^{1}H NMR). Data are reported as follows: chemical shift in ppm (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal), coupling constant (Hz), integration. High-resolution mass spectra (HRMS) were measured on a VG AutoSpec-Micromass spectrometer. Infrared spectra (IR) were obtained on a Thermo-Nicolet IR-200 spectrometer and absorptions are reported in reciprocal centimeters. HPLC analyses were performed with a Hewlett Packard HP-1100/HP-3395 equipment and optical rotations were measured with a Perkin–Elmer 341 polarimeter.

Compounds **1**,[20] **2**,[21] **13**[19] and **14**[19] have been described in the literature.

4.2. *N*-(Cbz)-4-Aryl-2-pyrroline 4a–d

To a solution of *N*-Cbz-3-pyrroline **2** (1.061 g; 5.2 mmol) in 36 mL of CH_3CN/H_2O (1:1; v/v) was added the arenediazonium salt (7.8 mmol) and 22 mg of $Pd(OAc)_2$ (0.1 mmol). The reaction mixture was stirred for 3 h at room temperature, when the total consumption of the substrate was observed by TLC analysis. The reaction mixture was then diluted with ethylacetate (~50 mL), transferred to a separatory funnel and extracted with saturated solutions of $NaHCO_3$ and NaCl. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and the solvent evaporated in vacuo, to give a dark brown oil (lactamol **3a–d**), which was submitted to the next reaction without further purification.

To a solution of lactamol **3a–d** (5.2 mmol) in 52 mL of anhydrous toluene was added 2,6-lutidine (3.0 mL; 26 mmol). To this mixture, at 0 °C, was slowly added 7.2 mL of a 0.7 M solution of trifluoroacetic anhydride (5.2 mmol). The ice bath was removed and the reaction mixture left under vigorous stirring for 2 h. The mixture was then heated at reflux for 30 min. After cooling to room temperature, the content was transferred to a separatory funnel containing 50 mL of ethyl acetate. The organic phase was washed with water and then with saturated solutions of $NaHCO_3$ and NaCl. The combined organic phases were dried over anhydrous sodium sulfate, filtered, and the solvent removed in vacuo to furnish a crude oil, which was purified by flash chromatography (eluent: hexane/ethyl acetate; 90:10; v/v), to provide the enecarbamates **4a–d** with yields ranging from 35% to 85% (over 2 steps).

Compond **4a** IR (cm^{-1}): 3115, 3093, 3066, 3039, 2963, 2897, 1719, 1627, 1496, 1420, 1339, 1219, 1132, 1089, 1024, 964, 915, 861 and 828. ^{1}H NMR (300 MHz, CCl_4/D_2O): $\delta = 7.3$ (m, 5H), 7.21 (d, $J = 8.4$ Hz, 2H), 7.08 (d, $J = 8.1$ Hz, 2H), 6.67 and 6.78 (s, 1H), 5.1 (m, 3H), 4.1 (m, 2H), 3.6 (m, 1H). ^{13}C NMR (75 MHz, CCl_4/D_2O): $\delta = 150.9$ and 150.2, 141.6, 135.8, 132.4, 131.0 and 129.8, 128.3, 127.5, 110.4 and 110.1, 66.6, 53.6, 47.6 and 46.3. Anal. Calcd for $C_{18}H_{16}ClNO_2$: C, 68.90; H, 5.14; N, 4.46. Found: C, 68.87; H, 5.16; N, 4.45.

Compond **4b** IR (cm^{-1}): 3111, 3091, 3072, 3043, 3008, 2955, 2906, 2832, 1714, 1626, 1523, 1430, 1352, 1245, 1181, 1123, 1095, 1034, 966, 839, 766 and 703. ^{1}H NMR (300 MHz, CCl_4/D_2O): $\delta = 7.22$ (m, 5H), 7.01 (d, $J = 8.1$ Hz, 2H), 6,71 (d, $J = 8.4$ Hz, 2H), 6.64 (s, 1H), 5.05 (m, 3H), 4.1 (m, 2H), 3.72 (s, 3H), 3.58 (m, 1H). ^{13}C NMR (75 MHz, CCl_4/D_2O): $\delta = 158.2$, 151.2 and 150.5, 136.3, 135.4, 130.4 and 129.3, 128.1–113.7, 111.7 and 111.3, 66.7 and 66.6, 54.6, 54.2 and 54.1, 47.7 and 46.4. Anal. Calcd for $C_{19}H_{19}NO_3$: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.75; H, 6.23; N, 4.54.

Compond **4c** IR (cm^{-1}): 3115, 3071, 3039, 2957, 2903, 1730, 1627, 1529, 1431, 1360, 1219, 1127, 986, 861, 763 and 703. ^{1}H NMR (300 MHz, CCl_4/D_2O): $\delta = 8.10$ (d, $J = 8.8$ Hz, 2H), 7.32 (d, $J = 8.8$ Hz, 2H), 7.27 (m, 5H), 6.83 and 6.75 (s, 1H), 5.10 (m, 3H), 4.2 (m, 2H), 3.6 (m, 1H). ^{13}C NMR (75 MHz, CCl_4/D_2O): $\delta = 151.1$, 150.3, 147.0, 135.9, 132.0 and 130.9, 128.2–127.6, 123.7, 109.7 and 109.4, 67.1, 53.6, 48.1 and 46.8. Anal. Calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.62; H, 4.95; N, 8.61.

Compond **4d** IR (cm^{-1}): 3047, 2955, 2891, 1719, 1616, 1499, 1426, 1347, 1216, 1132, 1098, 961, 898, 864, 825, 756 and 707. ^{1}H NMR (300 MHz, CCl_4/D_2O): $\delta = 7.8$–7.1 (m, 12H), 6.81 and 6.71 (s, 1H), 5.1 (m, 3H), 4.2 (m, 2H), 3.7 (m, 1H). ^{13}C NMR (75 MHz, CCl_4/D_2O): $\delta = 151.3$ and 150.6, 140.6, 136.2, 133.2, 132.3, 131.0 and 129.8, 128.5–125.1, 111.2 and 110.8, 66.80 and 66.65, 53.9, 48.6 and 47.3. Anal. Calcd for $C_{22}H_{19}NO_2$: C, 80.22; H, 5.81; N, 4.25. Found: C, 80.17; H, 5.79; N, 4.24.

4.3. (±)-*trans*-*N*-(Cbz)-3-Hydroxy-4-arylpyrrolidine 5a–d

To a solution of enecarbamate **4a**–**d** (1.1 mmol) in 11.5 mL of dry THF, under an inert atmosphere, at 0 °C, was added slowly $BH_3{\cdot}SMe_2$ (0.167 g; 0.21 mL; 2.2 mmol). The reaction mixture was then maintained at room temperature for 3 h. After total consumption of the substrate, the system was cooled again to 0 °C when 8.0 mL of a 3 M NaOH solution was added dropwise, followed by 8.0 mL of 30% H_2O_2. The mixture was stirred for 45 min in an ice bath followed by another 45 min at room temperature. After that, the mixture was transferred to a separatory funnel with 30 mL of ethyl acetate, the organic layer was washed with water, and then with saturated solutions of $NaHCO_3$ and NaCl. Finally, the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated in vacuo. The resultant oil was purified by flash chromatography (hexane/ethyl acetate; 60:40; v/v), to give alcohols **5a**–**d** with yields of 94%, 81%, 90% and 85%, respectively.

Compound **5a** IR (cm^{-1}): 3419, 3062, 3038, 2945, 2891, 1704, 1421, 1357, 1211, 1093, 1025, 971, 918, 830, 737 and 698. (3*S*,4*R*)-**5a**; $[\alpha]_D^{20} = +15.0$ (*c* 3.0, CH_2Cl_2) ee = 83.5%; (3*R*,4*S*)-**5a**; $[\alpha]_D^{20} = -19.0$ (*c* 3.0, CH_2Cl_2) ee = 96.9%; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.5$–7.1 (m, 9H), 5.1 (s, 2H), 4.27 (m, 1H), 3.93 (dd, $J = 11.3$ and 7.9 Hz, 1H), 3.77 (m, 1H), 3.55 (m, 1H), 3.37 (m, 1H), 3.24 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 154.8$, 137.5, 136.6, 133.1, 129.0–127.9, 76.5 and 75.6, 67.1, 52.4 and 52.0, 51.2 and 50.6, 49.9. HRMS (ESI): *m*/*z* calcd for $C_{18}H_{18}ClNO_3$+[H^+]: 332.1019; found: 332.1053. Anal. Calcd for $C_{18}H_{18}ClNO_3$: C, 65.15; H, 5.47; Cl, 10.69; N, 4.22. Found: C, 65.09; H, 5.35; Cl, 10.49; N, 4.19.

Compound **5b** IR (cm^{-1}): 3412, 3063, 3032, 2950, 2894, 2835, 1705, 1612, 1584, 1514, 1498, 1428, 1359, 1324, 1306, 1283, 1249, 1210, 1179, 1114, 1101, 1076, 1033, 960, 913, 831, 768, 738 and 698. (3*S*,4*R*)-**5b**; $[\alpha]_D^{20} = +6.4$ (*c* 1.6, CH_2Cl_2) ee = 96.3%; (3*R*,4*S*)-**5b**; $[\alpha]_D^{20} = -6.7$ (*c* 1.2; CH_2Cl_2) ee = 97.1%; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.4$–6.8 (m, 9H), 5.1 (s, 2H), 4.22 (m, 1H), 3.89 (dd, $J = 11.3$ and 7.7 Hz, 1H), 3.75 (s, 3H), 3.70 (m, 1H), 3.53 (m, 1H), 3.33 (dd, $J = 11.3$ and 5.5 Hz, 1H), 3.2 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 158.5$, 154.7, 136.6, 130.5, 130–127, 114.0, 76.5 and 75.7, 66.9, 55.2, 52.3 and 51.9, 51.0 and 50.4, 50.2. Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.53; H, 6.54; N, 4.20.

Compound **5c** IR (cm^{-1}): 3426, 3112, 3072, 3031, 2950, 2886, 1697, 1604, 1523, 1441, 1348, 1203, 1174, 1116, 971, 919, 861, 774, 751 and 704. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.16$ (d, $J = 8.5$ Hz, 2H), 7.5–7.3 (m, 7H), 5.15 (s, 2H), 4.35 (m, 1H), 3.99 (dd, $J = 11.4$ and 7.7 Hz, 1H), 3.80 (dd, $J = 11.3$ and 6.2 Hz, 1H), 3.62 (m, 1H), 3.4 (dd, $J = 11.4$ and 5.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 154.4$, 146.9, 146.5, 135.1, 128.3–127.7, 123.8, 76.2 and 75.3, 67.1, 52.4 and 52.1, 51.5 and 50.9, 49.56. Anal. Calcd for $C_{18}H_{18}N_2O_5$: C, 63.15; H, 5.30; N, 8.18. Found: C, 63.07; H, 5.43; N, 8.08.

Compound **5d** IR (cm^{-1}): 3414, 3062, 2950, 2886, 1704, 1680, 1601, 1430, 1367, 1323, 1220, 1181, 1098, 1074, 952, 918, 869, 825, 747 and 698. ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.8$–7.2 (m, 12H), 5.15 (s, 2H), 4.31 (m, 1H), 3.95 (m, 1H), 3.78 (m, 1H), 3.65 (m, 1H), 3.35 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 154.7$ and 154.6, 136.5 and 136.4, 136.2 and 136.1, 133.2, 132.3, 128.4–125.1, 76.2 and 75.3, 66.8, 52.2, 51.7 and 51.1, 49.8. Anal. Calcd for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C, 76.11; H, 6.20; N, 4.10.

4.4. (±)-*trans*-*N*-(Cbz)-3-Acetyl-4-aryl-pyrrolidine 6a–d

Alcohol substrate **5a**–**d** (1.0 mmol) was dissolved in 21.0 mL of dichloromethane. To this solution was added acetic anhydride (2.55 g; 2.3 mL; 25 mmol), pyridine (0.989 g; 1 mL; 12,5 mmol) and DMAP (12.2 mg; 0.1 mmol), and the reaction mixture magnetically stirred at room temperature for 2 h. Next, the solvent was removed in vacuo, and the residue was purified by flash chromatography to provide compounds **6a**–**d** in yields of 98%, 89%, 82% and 91%, respectively.

Compound **6a** IR (cm^{-1}): 3062, 3043, 2964, 2886, 1743, 1709, 1499, 1416, 1357, 1240, 1172, 1108, 1093, 1059, 1015, 986, 913, 825, 766 and 693. ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.5$–7.1 (m, 9H), 5.19 (d, $J = 7.6$ Hz, 2H), 5.1 (m, 1H), 3.88 (m, 1H), 3.77 (m, 1H), 3.71 (dd, $J = 11.3$ and 3.9 Hz, 1H), 3.53 (dd, $J = 12.9$ and 2.5 Hz, 1H), 3.46 (m, 1H), 2.1 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.4$ and 170.3, 154.6, 137.17 and 137.03, 136.53 and 136.47, 133.2, 129.1–127.9, 78.5 and 77.6, 67.1, 49.9 and 49.6, 49.15 and 49.11, 48.4 and 47.4, 21.0. HRMS (ESI): *m*/*z* calcd for $C_{20}H_{20}ClNO_4$+[H^+]: 374.1159; found: 374.1137.

Compound **6b** IR (cm^{-1}): 3071, 3033, 2957, 2897, 2843, 1746, 1708, 1621, 1588, 1523, 1426, 1355, 1241, 1181, 1111, 1062, 839, 768 and 698. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.5$–6.8 (m, 9H), 5.20 (d, $J = 4.0$ Hz, 2H), 5.13 (m, 1H), 3.8 (m, 6H), 3.5 (m, 2H), 2.1 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.2$, 158.6, 154.5, 136.5, 130.5 and 130.4, 128.3–127.7, 114.1, 78.7 and 77.9, 67.0, 55.2, 50.1 and 4.7, 49.4, 48.2 and 47.2, 21.1. HRMS (ESI): *m*/*z* calcd for $C_{21}H_{23}NO_5$+[H^+]: 370.1654; found: 370.1601.

Compound **6c** IR (cm^{-1}): 3119, 3074, 3035, 2956, 2895, 1745, 1711, 1616, 1543, 1419, 1363, 1234, 1111, 1066, 869, 757 and 707. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.19$ (d, $J = 8.5$ Hz, 2H), 7.4 (m, 7H), 5.20 (d, $J = 3.3$ Hz, 2H), 5.14 (m, 1H), 3.94 (m, 1H), 3.8 (m, 2H), 3,56 (m, 2H), 2.1 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.1$, 154.3, 147.1, 145.8, 136.2, 128.3–127.8, 124.0, 78.0 and 77.1, 67.2, 49.9 and 49.6, 48.9, 48.9 and 48.0, 20.9. HRMS (ESI): *m*/*z* calcd for $C_{20}H_{20}N_2O_5$+[H^+]: 384.13214; found: 384.1336.

Compound **6d** IR (cm^{-1}): 3066, 3035, 2959, 2887, 1744, 1708, 1606, 1422, 1361, 1320, 1243, 1177, 1116, 1059, 1024, 968, 922, 865, 830, 768, 753 and 707. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.9$–7.2 (m, 12H), 5.22 (m, 3H), 3.89 (m, 3H), 3.58 (m, 2H), 2.1 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.2$, 154.4, 136.4, 135.8 and

135.7, 133.1, 132.3, 128.5–125.3, 78.5 and 77.6, 67.0, 50.1 and 49.7, 49.2, 49.0 and 48.1, 21.1. HRMS (ESI): *m*/*z* calcd for $C_{24}H_{23}NO_4$+[H^+]: 389.16271; found: 389.16319.

4.5. Resolution of (±)-*trans*-*N*-(Cbz)-3-acetyl-4-aryl-pyrrolidine 6a–d (enzymatic hydrolysis)

To a round-bottomed reaction flask was added 50 mL of potassium phosphate buffer (pH 7.0), 5.0 mL of toluene, acetate **6a**–**d** (1.5 mmol) and 5.42 g of *Pseudomonas cepacia* AY lipase. The reaction was maintained at room temperature for 30 days under rigorous stirring. After this period, the flask contents were filtered through Celite. The organic phase was separated and washed with water and with saturated solutions of $NaHCO_3$ and NaCl. The solvent was removed in vacuo and the crude product purified by flash chromatography (hexane:ethyl acetate, 6:4; v/v), furnishing alcohols **5a**–**d** (98.8%, 96.3%, 92.9% and 97.2% ee, respectively) and the remaining acetates **6a**–**d** (58.0%, 97.1%, 93.0% and 99.4% ee, respectively).

4.6. (±)-*trans*-3-Acetyl-4-*p*-MeO-phenyl-pyrrolidine 7b

To a solution of acetate **6b** (0.75 mmol) in 22.5 mL of methanol was added palladium hydroxide dispersed over activated carbon (26.3 mg; 0.19 mmol). Hydrogen was purged through the resulting suspension for about 10 min under vigorous stirring, and then left under a balloon of hydrogen for 2 h. Next, the reaction was filtered through Celite, and the solvent evaporated in vacuo. The crude oil was then purified by flash chromatography ($CHCl_3$/MeOH, 9:1, v/v), to yield free amine **7b** in 88%. Compound **7b** IR (cm^{-1}): 3321, 2959, 2939, 2865, 2830, 1740, 1646, 1621, 1517, 1423, 1378, 1249, 1190, 1036, 992 and 833. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.15$ (d, $J = 8.7$, 2H), 6.85 (d, $J = 8.4$ Hz, 2H), 5.14 (ddd, $J = 5.9$, 3.7 and 2.6 Hz, 1H), 3.78 (s, 3H), 3.50 (dd, $J = 11.3$ and 7.7 Hz, 1H), 3.27 (m, 2H), 3.08 (dd, $J = 12.8$ and 2.2 Hz, 1H), 2.91 (dd, $J = 11.3$ and 7.7 Hz, 1H); 2.53 (s, 1H), 2.05 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.5$, 158.2, 132.9, 128.1, 113.9, 82.3, 55.2, 53.9, 53.4, 51.2, 21.2. Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.32; H, 7.25; N, 5.93.

4.7. (±)-*trans*-3-Acetyl-4-*p*-MeO-phenyl-pyrrolidinium iodide 8b

A mixture of free amine **7b** (0.24 mmol), methyl iodide (1.7 g; 0.75 mL; 12 mmol), sodium bicarbonate (0.101 g; 1.2 mmol) in 10.0 mL of anhydrous dichloromethane was heated at reflux under inert atmosphere for 4 days. After the consumption of amine **7b** (TLC), the solvent was removed in vacuo to give a crude oil, which was purified by flash chromatography ($CHCl_3$/MeOH, 8:2, v/v), providing the quaternary ammonium salt **8b** in quantitative yield.

Compound **8b** IR (cm^{-1}): 3425, 2924, 1738, 1614, 1517, 1458, 1253, 1181, 1026. (3*S*,4*R*)-**8b**; $[\alpha]_D^{20} = -13.5$ (*c* 1.3, H_2O) ee = 96.3%; (3*R*,4*S*)-**8b**; $[\alpha]_D^{20} = +17.5$ (*c* 2.0, H_2O) ee = 97.1%; ^{1}H NMR (300 MHz, D_2O): $\delta = 7.25$ (d, $J = 9.2$ Hz, 2H), 6.92 (d, $J = 8.8$ Hz, 2H), 5.40 (m, 1H), 3.7 (s, 3H), 4.2–3.5 (m, 5H), 3.29 (s, 3H), 3.26 (s, 3H), 2.0 (s, 3H). ^{13}C NMR (75 MHz, D_2O/CCl_4): $\delta = 172.5$, 158.14, 128,6–128.3, 114.3, 77.9, 69.6 55.3, 53.4, 52.5, 47.9, 20.1. (ESI): *m*/*z* calcd for $C_{15}H_{22}NO_3$+[H^+]: 264.1600; found: 264.1574.

4.8. Pyrrolidine-(3*R*,4*S*)-methoxyphenylacetate 11 and pyrrolidine-(3*S*,4*S*)-methoxyphenylacetate 12

To a solution of the resolved alcohols (−)-(3*R*,4*S*)-**5b** (0.060 g; 0.2 mmol) or (+)-(3*S*,4*R*)-**5b** (0.046 g; 0.1 mmol) in 5.0 mL of dichloromethane was added methoxyphenylacetic acid (MPA; 1 equiv) and catalytic amounts of DMAP (2 mol %), and the mixture was left stirring for 3 h. The reaction mixture was then filtered to remove solid side products (mostly dicyclohexylurea). The filtrate was evaporated in vacuo and the residue purified by flash chromatography (hexane:ethyl acetate; 6:4; v/v), furnishing the respective esters (75% yield). Deprotection of the esters were conducted as described in item 4.5 to give diastereomeric free-amines (*RSS*)-**11** and (*SRS*)-**12** in 40% yield.

(*RSS*)-**11** IR (cm^{-1}): 3362, 3059, 3046, 2932, 2844, 1756, 1621, 1514, 1460, 1252, 1191, 1124, 1030, 828, 734 and 701. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.5$–7.3 (m, 5H), 6.97 (d, $J = 8.5$ Hz, 2H), 6.77 (d, $J = 8.9$ Hz, 2H), 5.14 (ddd, $J = 5.8$, 3.3 and 2.1 Hz, 1H), 3.8 (s, 3H), 3.4 (s, 3H), 3.35 (dd, $J = 11.3$ and 7.6 Hz, 1H), 3.26 (dd, $J = 12.8$ and 5.8 Hz, 1H), 3.08 (td, $J = 7.6$ and 3.9 Hz, 1H), 3.06 (dd, $J = 12.8$ and 2.1 Hz, 1H), 2.9 (dd, $J = 11.3$ and 7.3 Hz, 1H), 1.9 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.0$, 158.1, 135.8, 132.4, 128.5–126.8, 113.8, 83.1, 82.3, 57.2, 55.1, 53.6, 53.0, 51.1. HRMS (ESI): *m*/*z* calcd for $C_{20}H_{23}NO_4$+[H^+]: 342.1705; found: 342.1696.

(*SRS*)-**12** IR (cm^{-1}): 3381, 3069, 3051, 2943, 2847, 1759, 1615, 1519, 1453, 1249, 1183, 1111, 1033, 828, 738 and 702. ^{1}H NMR (500 MHz $CDCl_3$): $\delta = 7.4$–7.3 (m, 5H), 7.11 (d, $J = 8.6$ Hz, 2H), 6.83 (d, $J = 8.8$ Hz, 2H), 5.181 (ddd, $J = 5.5$, 3.4 and 2.1 Hz, 1H), 3.44 (dd, $J = 11.3$ and 7.9 Hz, 1H), 3.25 (td, $J = 7.3$ and 3.3 Hz, 1H), 3.18 (dd, $J = 13.2$ and 5.5 Hz, 1H), 2.89 (dd, $J = 11.6$ and 7.3 Hz, 1H), 2.84 (dd, $J = 13.2$ and 1.9 Hz, 1H), 1.9 (1H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.4$, 158.4, 136.1, 132.7, 128.9–127.1, 114.1, 83.3, 82.5, 57.3, 55.3, 53.8, 53.2, 51.2. HRMS (ESI): *m*/*z* calcd for $C_{20}H_{23}NO_4$+[H^+]: 342.1705; found: 342.1698.

4.9. Resolution of (±)-*N*-(Cbz)-3-hydroxy-pyrrolidine 13 (enzymatic transesterification)

To a solution of racemic alcohol (±)-**13** (0.518 g; 2.3 mmol) in 25 mL of dichloromethane it was added vinyl acetate (0.198 g; 2.1 mL; 23 mmol) and the immobilized lipase (*Pseudomonas cepacia* AK). The resulting suspension was maintained under vigorous stirring at room temperature for 10 days. After this period, the mixture was filtered through a synterized funnel and the solvent evaporated in vacuo. The crude oil was then purified by flash chromatography (hexane:ethyl acetate; 6:4; v/v), providing 0.262 g of the acetylated compound (3*R*)-**14** (>99% ee) and 0.280 g of alcohol (3*S*)-**13** (81.3% ee). (3*R*)-**14**; $[\alpha]_D^{20} = -13.5$ (*c* 1.48; CH_3OH) ee >99%.

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