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Enzymatic resolution of Indinavir precursor in ionic liquids with reuse of biocatalyst and media by product sublimation

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Experimental

General

All reagents were obtained commercially unless otherwise noted. Thin layer chromatography was performed on aluminium-backed silica gel Merck 60 F_{254} plates. Visualization of developed chromatogram was accomplished by fluorescence (254 nm lamp) and by staining with ethanolic phosphomolybdic acid or aqueous potassium permanganate solution. Column chromatography was performed using Silica gel 60 and was used for analytical TLC. Nuclear magnetic resonance (NMR) ^{1}H (400.13 MHz) and ^{13}C (100.61 MHz), spectra were recorded on a Bruker AMX 400 Spectrometer. Chemical shifts are reported downfield in parts per million (ppm) from tetramethylsilane reference. IR spectra were recorded on a Jasco FTIR as film on NaCl or KBr plates. Melting points (uncorrected) were determined on an Electrothermal Mod. IA6304 capillary melting point apparatus. HPLC analysis were performed using Dionex HPLC components UVD340S, P680, using Chiralcel OD guard column(0.46 cm ∅, 5 cm) and Chiralcel OD column (0.46 cm ∅, 25 cm) at 25ºC, λ 216 and 263nm.

Immobilized *Candida antarctica* lipase B (Novozym 435® with 1-2% water w/w and 7000 PLU/g) was a gift from Novo Nordisk Bioindustrial, Spain. *Pseudomonas cepacia* lipase was a commercial preparation from Fluka.

The ionic liquids (ILs) based on 1-*n*-butyl-3-methylimidazolium were a gift from Solchemar.[1] Aliquat 336® was purchased from Aldrich (2:1 mixture of C_8 and C_{10} chains).[2]

[Aliquat][N(CN)$_2$]- Tricaprylmethylammonium dicyanamide:

To a stirred solution of Aliquat 336® (8.47g; 20.9 mmol) (2:1 mixture of C_8 and C_{10} chains) in 10 mL CH_2Cl_2 was added sodium dicyanamide (2.23 g; 25.1 mmol). The mixture was stirred for 48 h at room temperature. The precipitate was then filtered and washed with CH_2Cl_2, after which the solvent was removed under vacuum overnight at room temperature to afford [Aliquat] [$N(CN)_2$] in 75% yield as a colourless oil; water content: 3.3 µg H_2O/ µL ionic liquid.

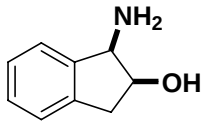
^{1}H NMR ($CDCl_3$) δ 0.86 (t, 9H, *J*=5.2Hz, CH_3), 1.25-1.35 (m, 42H, $(CH_2)_7$-CH_3), 1.68 (br s, 6H, CH_2), 3.12 (s, 3H), 3.27 (m, 6H).

^{13}C NMR ($CDCl_3$) δ 14.10, 22.20, 22.77, 26.31, 26.45, 29.10, 29.19, 29.51, 29.58, 29.70, 29.80, 29.96, 31.90, 32.08, 47.79, 61.57, 61.47, 119.73.

IR (NaCl) 2958, 2927, 2854, 2233, 2133(CN), 1466, 1311 cm^{-1}.

Anal. Calcd. for $C_{27}H_{54}N_4$: C, 74.59; H, 12.52; N, 12.89. Found C, 75.04; H, 12.72; N, 11.94.

Racemic *cis*-1 amino-2-indanol (±)1:

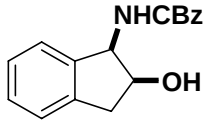


(±) **1**

This compound was prepared by the Ritter reaction using *trans*-2-bromo-1-indanol as starting material and following the reported procedure.[3]

^{1}H NMR ($CDCl_3$) δ 1.62 (reported[4] as 2.30 br s, 2H, NH_2), 2.94 (dd, 1H, *J*=2.6, 16.4Hz, CH_2), 3.09 (dd, 1H, *J*=5.5, 16.4Hz, CH_2), 4.31 (d, 1H, *J*=4.4Hz, CH), 4.40-4.36 (m, 1H, CH), 7.29-7.23 (m, 4H, H ar.).
^{1}H NMR spectra was in good agreement with literature data.[4]

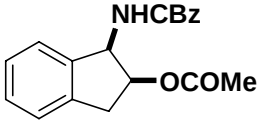
Racemic *cis*-benzyl N-(1-hydroxyindan-2-yl)carbamate (±) 2:



(±) **2**

To a stirred solution of (±) *cis*-1-amino-2-indanol (±) **1** (5.0 g; 35.5 mmol) and sodium carbonate (6.86 g; 40.2 mmol) in 60 mL water at 0 ºC was added benzyl chloroformate (4.27 g; 40.2 mmol) drop wise over 30 min. The reaction mixture was then allowed to warm to room temperature over 6 h. The reaction mixture was then extracted with CH_2Cl_2 (3×20 mL). The combined organic phases were dried ($MgSO_4$) and filtered. The solvent was removed under vacuum leaving a solid that after purification by flash column chromatography (SiO_2 20% EtOAc/ 80% hexane) gave **(±) 2** as a white solid. m.p. 139-141ºC (lit.[5] 140-142ºC).
^{1}H NMR ($CDCl_3$) δ 2.06 (br s, 1H, OH), 2.90 (d, 1H, *J*=16.4Hz, CH_2), 3.12 (dd, 1H, *J*=4.5, 16.5 Hz, CH_2), 4.58 (br s, 1H, CH), 5.08-5.20 (m, 3H, OCH_2Ph, CH), 5.48 (d, 1H, *J*=6.8 Hz, NH), 7.24-7.39 (m, 9H ar.)
^{13}C NMR ($CDCl_3$) 39.5 (CH_2), 59.3 (CH), 67.1(CH_2), 73.6(CH), 124.46(CH ar.), 125.4 (CH ar.), 127.2 (CH ar.), 128.2 (CH ar.), 128.3 (CH ar.), 128.6 (CH ar.), 136.3 (C ar.), 139.7 (C ar.), 140.4 (C ar.), 156.8 (CO).
IR (KBr) 3473, 3338, 1666, 1550, 1254, 1057 cm^{-1}.
HPLC (Chiracel OD, hexane:isopropanol (95:5), 1 mL/min, λ 263nm.); t_R (*1S*, 2*R*)-**2** = 31.91 min; t_R (*1R*,2*S*)-**2** = 36.67 min.
^{1}H and ^{13}C NMR spectra were in good agreement with literature data.[5]

Racemic *cis*-benzyl N-(1-acetoxyindan-2-yl)carbamate (±) 3:

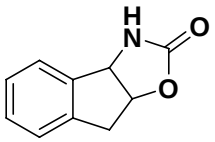


(±) **3**

To a stirred solution of (±) *cis*-benzyl N-(1-hydroxyindan-2-yl)carbamate (±) **2** (175 mg; 0.62 mmol) in 5 mL of anhydrous CH_2Cl_2 at 0 ºC, under argon atmosphere was added triethylamine (172 µL; 1.2 mmol). Acetyl chloride (66 µL, 0.93 mmol) was then added drop wise to the reaction mixture. The reaction mixture was allowed to warm to room temperature overnight, after which was quenched with aqueous $NaHCO_3$ and the organic layer was extracted with HCl 10% (3×10 mL). The combined organic phases were dried ($MgSO_4$) and filtered. The solvent was removed under vacuum leaving a solid. Purification by flash column chromatography (SiO_2 10% EtOAc/ 90% hexane) gave **(±) 3** as a white solid. m.p. 123-125 ºC. [lit.[5] 147-149 ºC for (1*S*, 2*R*) *cis*-benzyl N-(1-acetoxyindan-2-yl)carbamate; (1*S*, 2*R*) **3**].
^{1}H NMR ($CDCl_3$) δ 1.90 (s, 3H, CH_3), 2.93 (d, 1H, *J*=17.1Hz, CH_2), 3.13 (dd, 1H, *J*=4.5, 17.0 Hz, CH_2), 5.10 (s, 2H, CH_2Ph), 5.35 (m, 1H), 5.50 (m, 1H, CHOAc), 7.16-7.32 (m, 9H ar.)

^{13}C NMR ($CDCl_3$) 21.0 (CH), 37.3 (CH), 57.4 (CH), 67.1 (CH), 75.6 (CH), 123.8(CH ar.), 125.0 (CH ar.), 127.3 (CH ar.), 128.2 (CH ar.), 128.4 (CH ar.), 128.5 (CH ar.), 136.3 (C ar.), 139.2 (C ar.), 140.4 (C ar.), 156.2 (CO carbamate), 170.3 (CO ester).
IR (KBr) 3305, 1732, 1701, 1535, 1265 cm^{-1}.
HPLC (Chiracel OD, hexane:isopropanol (95:5), 1 mL/min, λ 263nm.); t_R **3** = 24.02 min. single peak.

Racemic Indanol[1,2-d]oxazolidin 2-one (±) 4:



(±) **4**

To a stirred solution of (±) *cis*-benzyl N-(1-hydroxyindan-2-yl)carbamate (±) **2** (100 mg; 0.36 mmol) in 2 mL of methanol at room temperature was added NaOH (43 mg; 1.1mmol). The reaction mixture was stirred for 2 h, after which was quenched with aqueous NH_4Cl and extraction with AcOEt (3×25 mL) was carried out. The combined organic phases where dried ($MgSO_4$) and filtered. The solvent was then removed under vacuum leaving a white solid. Purification by flash column chromatography (SiO_2 10-30% EtOAc/ hexane) gave **(±) 4** as a white solid which was recr**y**stalized in AcOEt/hexane. m.p.149-150ºC (lit.[6] 205-207ºC for (4*R*,5*S*)-indanol[1,2-d]oxazolidin 2-one).
^{1}H NMR ($CDCl_3$) δ 3.33-3.42 (m, 2H, CH_2), 5.15 (d, 1H, *J*=7.3 Hz, CH), 5.37-5.41 (m, 1H, CH), 6.9 (br s, 1H, NH), 7.20-7.32 (m, 4H ar.)
^{13}C NMR ($CDCl_3$) 38.8 (CH_2), 61.2 (CH), 80.6(CH_2), 124.7(CH ar.), 125.5 (CH ar.), 127.9 (CH ar.), 129.3 (CH ar.), 139.7 (CH ar.), 140.2 (CH ar.), 160.7 (CO).
IR (KBr) 3255, 2922, 1752, 1705, 1481, 1392, 1230, 1053, 752 cm^{-1}
Anal. Calcd. for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00; O, 18.27. Found: C, 68.16; H, 5.20; N, 8.07
^{1}H and ^{13}CNMR spectra were in good agreement with literature data.[6,7]

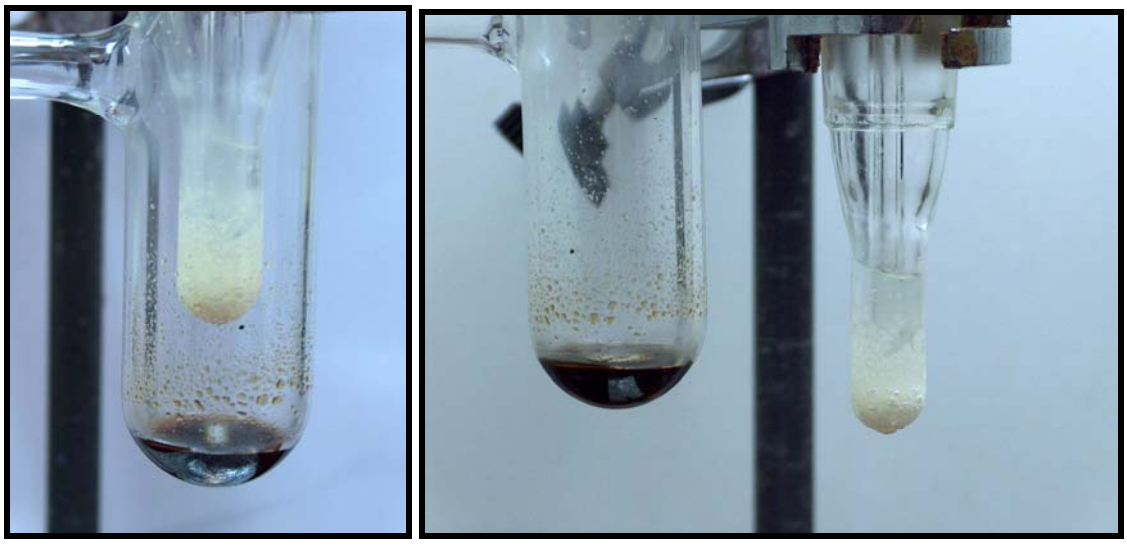
Screening of ionic liquids for the enzymatic resolution of (±)*cis*-benzyl N-(1-hydroxyindan-2-yl)carbamate (±) 2 (Table 1):
To a stirred solution of (±) *cis*-benzyl N-(1-hydroxyindan-2-yl)carbamate (±) **2** (20 mg, 0.07 mmol) in 0.5 mL of ionic liquid (equilibrated through the vapour phase with a saturated solution of potassium acetate for ca. 3 days under magnetic stirring at 25ºC to provide an water activity a_w=0.2[8]) at 35ºC in a thermostatic bath was added Novozym 435® (20 mg) and vinyl acetate (0.71 mmol, 65 µL). Portion of crude mixture was taken at several times (24 h, 72 h), diluted in Et_2O and immobilized onto silica gel by removing the Et_2O under vacuum, filtered through a silica pipette size column with Et_2O (10mL) and analysed by HPLC (Chiracel OD, hexane:isopropanol (95:5), 1 mL/min, λ 263nm.). The optical purity of **3** was calculated on the basis of the observed conversion and ee of **2** by HPLC. The calculated ee value of **3** is on average 4% lower than the ee observed for derived isolated product **2** (measured after hydrolysis). The reaction was left running for 60 h, after which the enzyme was filtered off and washed with Et_2O (10mL).

Procedure for the enzymatic resolution of (±)*cis*-benzyl N-(1-hydroxyindan-2-yl)carbamate (±) 2 (Table 2):
To a stirred solution of (±) *cis*-benzyl N-(1-hydroxyindan-2-yl)carbamate (±) **2** (100 mg, 0.36 mmol) in 1 mL of [aliq][$N(CN)_2$] (water content of 3.3 µg H_2O/ µL ionic liquid) at 25 ºC in a thermostatic bath was added Novozym 435® (100 mg) and vinyl acetate (35.5 mmol, 327 µL). Portion of crude mixture was taken at several times (24 h,

60 h), diluted with Et_2O and immobilized onto silica gel by removing the Et_2O under vacuum, filtered through a silica pipette size column with Et_2O (10mL) and analysed by HPLC. The optical purity of **3** was calculated on the basis of the observed conversion and ee of **2** by HPLC. The calculated ee value of **3** is on average 4% lower than the ee observed for derived isolated product **2** (measured after hydrolysis). The reaction was left running for 60 h, after which the enzyme was filtered off and washed with Et_2O (10 mL).

After 1 day of reaction a conversion of 31% was obtained for benzyl (1*S*, 2*R*)N-(1-acetoxyindan-2-yl)carbamate (1*S*, 2*R*) **3** and an e.e of 40% for (1*R*, 2*S*) benzyl N-(1-hydroxyindan-2-yl)carbamate (1*R*, 2*S*) **2** was measured by HPLC (Chiracel OD, hexane:isopropanol (95:5), 1 mL/min, λ 263 nm.). The compounds were isolated by flash column chromatography (SiO_2 20% EtOAc/ 80% hexane) providing by order of elution: Benzyl (1*S*, 2*R*)N-(1-acetoxyindan-2-yl)carbamate (1*S*, 2*R*) **3** as white solid (32.2 mg, 28 %, e.e 97 %; measured after hydrolysis, see bellow) and (1*R*, 2*S*) benzyl N-(1-hydroxyindan-2-yl)carbamate (1*R*, 2*S*) **2** as a white solid (67.4 mg, 67 %, e.e 40%).

For another similar reaction, after 2.5 days a conversion of 48% was obtained for benzyl (1*S*, 2*R*)N-(1-acetoxyindan-2-yl)carbamate (1*S*, 2*R*) **3** and a e.e of 80% was measured for (1*R*, 2*S*) Benzyl N-(1-hydroxyindan-2-yl)carbamate (1*R*, 2*S*) **2**. The remaining part of the reaction mixture was submitted to sublimation under high vacuum provided with a diffusion pump (2h, 130-140ºC, 3×10^{-5} mbar), giving a mixture of compounds (Figure ESI-1) that were further purified by flash column chromatography (SiO_2 20% EtOAc/ 80% hexane) giving, by order of elution: Benzyl (1*S*, 2*R*)N-(1-acetoxyindan-2-yl)carbamate (1*S*, 2*R*) **3** as a white solid (41 mg, 36%, e.e 92%; measured after hydrolysis, see bellow), (1*R*, 2*S*) benzyl N-(1-hydroxyindan-2-yl)carbamate (1*R*, 2*S*) **2** as a white solid (5,1 mg, 5%, e.e 80%) and indanol[1,2-d]oxazolidin 2-one **4** as a white solid (26,2 mg, 42%).



a) **b)**

Figure ESI-1. Picture showing the sublimation of **2** and **3** just before (a) and right after (b) disconnecting the system.

Procedure for recycling the enzymatic resolution of (±)*cis*-benzyl N-(1-hydroxyindan-2-yl)carbamate (±) 2:

To a stirred solution of (±) *cis*-benzyl N-(1-hydroxyindan-2-yl)carbamate (±) 2 (100 mg, 0.355 mmol) in 1 mL of [aliq][$N(CN)_2$] (recovered from previous reaction, Table 2,

entry 3) at 25 ºC in a thermostatic bath was added Novozym 435® (aprox. 100 mg recovered from previous reaction, Table 2, entry 3) and vinyl acetate (35.5 mmol, 327 µL). The reaction was left running for 60 h, after which the enzyme was filtered off and washed with Et_2O (10 mL). Portion of crude reaction was taken at several times, diluted in Et_2O and immobilized onto silica gel by removing the Et_2O under vacuum, filtered through a silica pipette size column with Et_2O (10 mL) and analysed by HPLC. The reaction conversion was 44 % for benzyl (1*S*, 2*R*)N-(1-acetoxyindan-2-yl)carbamate (1*S*, 2*R*) **3** and a ee of 65 % was obtained for (1*R*, 2*S*) benzyl N-(1-hydroxyindan-2-yl)carbamate (1*R*, 2*S*) **2**. The compounds were isolated by flash column chromatography (SiO_2 20% EtOAc/ 80% hexane) that afforded, by order of elution: Benzyl (1*S*, 2*R*)N-(1-acetoxyindan-2-yl)carbamate (1*S*, 2*R*) **3** as a white solid (46.6 mg, 40%, ee 88%; measured after hydrolysis, see bellow) and (1*R*, 2*S*) benzyl N-(1-hydroxyindan-2-yl)carbamate (1*R*, 2*S*) **2** as a white solid (56.1 mg, 55%, e.e 65%).

General procedure for the hydrolysis of (1*S*, 2*R*) *cis*-benzyl N-(1-acetoxyindan-2-yl)carbamate (1*S*, 2*R*) 3:

To a stirred solution of (1*S*, 2*R*) *cis* benzyl –N-(1-acetoxyindan-2-yl)carbamate, (1*S*, 2*R*) **3** (28.5 mg, 80.8 mmol) in 2 mL methanol at 0 ºC was added drop wise over 30 min a solution of NaOH/MeOH (1 mL, 91 mM). The reaction mixture was stirred for 1 h, after which was quenched with aqueous NH_4Cl and extraction with AcOEt (3×25 mL) was carried out. The combined organic phases were dried ($MgSO_4$) and filtered. The solvent was then removed under vacuum leaving a white solid. The compounds were isolated by flash column chromatography (SiO_2 20% EtOAc/ 80% hexane) that afforded, by order of elution: (1*S*, 2*R*) Benzyl N-(1-hydroxyindan-2-yl)carbamate (1*S*, 2*R*) **2** as white needles (24mg, 70%, e.e 92%), indanol[1,2-d]oxazolidin 2-one **4** as white solid (6.0 mg, 28%).

References

1. http://www.solchemar.com
2. http://www.phasetransfer.com/05tipJan.htm
3. Y. Igarashi, S. Otsutomo, M. Harada, S. Nakano and S. Watanabe, *Synthesis*, 1997, 549-552.
4. W. J. Thompson, P. M. D. Fitzgerald, M. K. Holloway, E. A. Emini, P. L. Darke, B. M. McKeever, W. A. Schleif, J. C. Quintero, J. A. Zugay, T. J. Tucker, J. E. Schwering, C. F. Homnick, J. Nunberg, J. P. Springer and J. R. Huff, *J. Med. Chem.*, 1992, **35**, 1685-1701.
5. A. Luna, A. Maestro, C. Astorga and V. Gotor, *Tetrahedron-Asymmetry*, 1999, **10**, 1969-1977.
6. A. K. Ghosh, J. F. Kincaid and M. G. Haske, *Synthesis*, 1997, 541-544
7. M. Suzuki, C. Nagasawa and T. Sugai, *Tetrahedron*, 2001, **57**, 4841-4848.
8. L. Greenspan, *J. Research Nat. Bur. Stand. – A. Phys. and Chem.*, 1977, 81A, 89-96.