Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 3251–3256

TETRAHEDRON: *ASYMMETRY*

An efficient chemoenzymatic approach to enantiomerically pure 4-[2-(difluoromethoxy)phenyl] substituted 1,4-dihydropyridine-3,5-dicarboxylates

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Received 14 November 2001; accepted 14 January 2002

Abstract—An efficient chemoenzymatic synthesis of (−)-3-methyl 5-(2-propoxyethyl) (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate has been achieved. The key step is a highly stereoselective *Candida rugosa* lipase (CRL)-mediated asymmetrisation of the prochiral bis[(isobutyryloxy)methyl]-4-[2-(difluoromethoxy)phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate.

1. Introduction

1,4-Dihydropyridines (1,4-DHPs) remain important because of their wide spectrum of biological activities, these include antidiabetic, nootropic, neuromodulatory, cognition and memory enhancing, neuroprotective (at age-related neuronal damage, anti-hypoxic, anti-alcohol) properties, regulatory mode action and neuropeptide effects.[1,2] When substituents on the left side differ from those on the right side of a 1,4-DHP, the molecule is chiral, with C(4) as the stereogenic centre. The enantiomers of an unsymmetrical 1,4-DHP usually differ in their biological activities and could even have an exactly opposite activity profile. Chiral 4-aryl-1,4-DHPs have been the subject of extensive investigation as calcium antagonists for the last two decades[3–8] and the chemoenzymatic synthesis of chiral 1,4-DHPs has been reported by several research groups, including us.[9–14] Herein, we present the *Candida rugosa* lipase-catalysed enantioselective hydrolysis of bifunctional prochiral bis [(isobutyryloxy)methyl] 4-[2-(difluoromethoxy)phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate **3**, which is a building block for the synthesis of potentially biologically active substances. Compound **3** was used to prepare (−)-3-methyl 5-(2-propoxyethyl) (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, which is an asymmetric analogue of cerebrocrast, a compound with cognition and memory enhancing, neuroprotective properties.[1,2]

2. Results and discussion

The main task of our studies was to reach the best enantioselectivity for the enzyme-mediated hydrolysis of prochiral 4-[2-(difluoromethoxy)phenyl] substituted dihydropyridines. Since ester groups which are directly attached to the heterocyclic ring are not cleaved by most enzymes, the readily cleavable acyloxymethyl esters were introduced at the 3 and 5 positions of the 1,4-DHP. It has been observed previously[14,15] that the size of the transformed acyloxymethyl ester group of a 1,4-DHP exerts a significant influence on the enantioselectivity of the enzymatic hydrolysis. The enantioselectivity of CRL-mediated hydrolysis increased together with the steric hindrance of the acyloxymethyl ester group and the highest enantiomeric ratio ($E=21$) was reached for racemic 3-[(isobutyryloxy)methyl] 5-methyl 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate.[14] For this reason, the isobutyryloxymethyl and pivaloyloxymethyl derivatives **3a** and **3b** were synthesised.

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0957-4166/01/$ - see front matter
PII: S0957-4166(02)00022-8

Substrates **3a** and **3b** were prepared in a three-step sequence as depicted in Scheme 1. Bis cyanoethyl ester **1** was synthesised starting from 2-cyanoethyl acetoacetate, 2-(difluoromethoxy)benzaldehyde and ammonia by a Hantzsch cyclisation in 38% yield. The hydrolysis of the diester **1** with KOH gave dicarboxylic acid **2** in 73% yield. The substrate **3a** was obtained by the treatment of **2** with pivaloyloxymethyl (POM) chloride in the presence of K_2CO_3 in 57% yield. Compound **3b** was obtained by the treatment of the in situ generated diacid **2** with isobutyryloxymethyl (*i*-BOM) chloride in 48% yield.

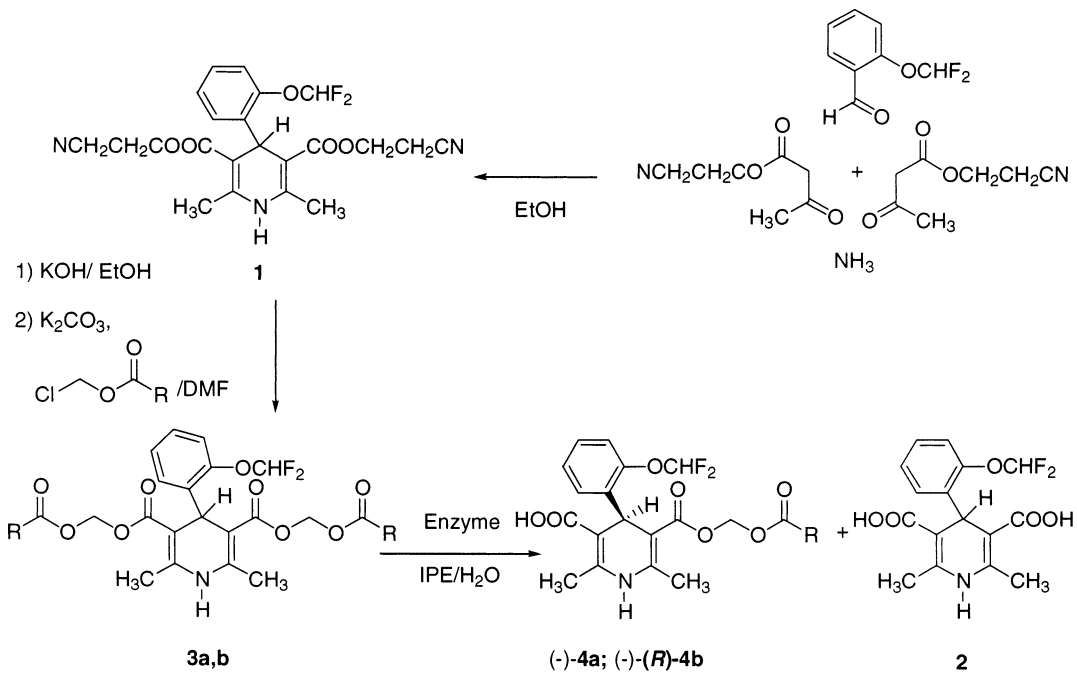
The bis POM ester **3a** was first tested for hydrolysis with lipase AH, as this enzyme has been used for the asymmetrisation of its 4-(3-nitrophenyl) analogue.[16] The hydrolysis of **3a** with lipase AH in aqueous media at 45°C occurred with formation of **4a** in 25% e.e. When *Candida rugosa* lipase (CRL) was applied for the hydrolysis of **3a** in water-saturated diisopropyl ether (IPE) at 45°C, the e.e. of monoacid **4a** was not constant and increased from 45% (25% conversion after 1 day) until 84% of e.e. (34% of conversion in 9 days), together with concomitant hydrolysis to the diacid **2** (9% in 9 days). Apparently, the enantiomeric excess of **4a** was improved by the CRL-catalysed kinetic resolution of enantiomerically enriched **4a** to achiral diacid **2**, as the second hydrolytic step.[17,18] Longer reaction time led to higher enantiomeric purity but also to a lower chemical yield of the product **4a** and therefore this method is not practical.

A better way to asymmetrise **3** involves hydrolysis of the bis *i*-BOM ester **3b**, which could be performed using *Candida rugosa* lipase as a catalyst in water-saturated IPE at rt, as shown in Scheme 1. It is necessary to monitor the reaction carefully by HPLC because of the subsequent hydrolysis to the diacid **2**. The reaction was interrupted when the ratio between the substrate, diacid **2** and **4b** was about 1:1:3. The enantioselectivity of the reaction was excellent (>95%).

The enantiomeric excess of **4b** was determined by an enantioselective HPLC method using a mixture of (+)-**4b** and (−)-**4b** as a reference standard. This (almost racemic) reference standard was prepared by hydrolysis of **3b** with the lipases of *Rhizomucor miehei* and *Candida antarctica* B. The first enzyme gave **4b** with 11% e.e., whereas *Candida antarctica* lipase B gave **4b** in 13% e.e. with opposite stereopreference.

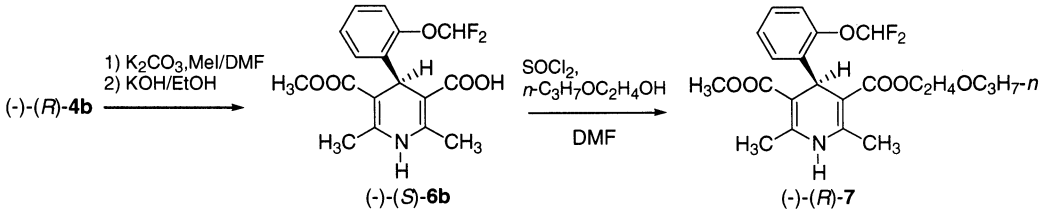
The monoacid (−)-(*R*)-**4b** was converted into the corresponding methyl ester (−)-(*R*)-**5b** by esterification with MeI in DMF with 87% yield (Scheme 2). The asymmetric analogue of Cerebrocrast (−)-(*R*)-**7** was obtained after removal of *i*-BOM group, treatment with $SOCl_2$, and a subsequent reaction with 2-propoxyethanol (propylcellosolve®) as reported before.[14]

The absolute configuration of (−)-**4b** was proven to be *R*. It was determined by the conversion of (−)-**4b** to (−)-**5b**, (−)-**6** and (−)-**7** which showed the same sign of optical rotation as that of (−)-(*R*)-**5b**, (−)-(*S*)-**6** and (−)-(*R*)-**7** as reported earlier.[14] The absolute configuration was also proven by the coupling of (−)-(*S*)-**6** with (*R*)-(α)-methylbenzylamine and comparing their order of elution on a reversed phase HPLC column with the literature data.[14]



a) R = $C(CH_3)_3$ (POM)
b) R = $CH(CH_3)_2$ (*i*-BOM)

Scheme 1.



Scheme 2.

CRL lipase prefers to hydrolyse the *pro*-*R* ester group of substrate **3b** which is in agreement with previously obtained data, in which this enzyme reacts preferentially with the *S*-form of the racemic mixture of unsymmetrical analogues of compound **3**, producing *R*-monoacids.[14]

3. Conclusion

The present study demonstrates the usefulness of *Candida rugosa* lipase for the asymmetrisation of **3b** with excellent enantiomeric purity. The enantioselectivity of the CRL-catalysed asymmetrisation of prochiral bifunctional substrates **3a** and **3b** to the monoacids **4a** and **4b** was enhanced by the second hydrolysis to achiral diacid **2** which is highly stereoselective. The synthesis of (−)-3-methyl 5-(2-propoxyethyl) (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (−)-(*R*)-**7** with ≥99% e.e. was achieved. The synthesis of other potentially biologically active chiral dihydropyridine derivatives using the building block (−)-(*R*)-**4b** is currently underway.

4. Experimental

4.1. General

All reagents were purchased from Aldrich, Acros or Merck and used without further purification. HPLC grade solvents were from Labscan (Dublin, Ireland). Flash column chromatography was performed on Merck silica gel 60 (230–400 mesh or 70–230 mesh). Preparative TLC was performed on 20×20 cm silica gel TLC-PET F_{254} foils (Fluka). *Candida rugosa* lipase (lipase (EC 3.1.1.3) Type VII from *Candida rugosa*) was purchased from Sigma. Lipase AH was supplied by Amano Pharmaceutical Co., Ltd (Japan). Immobilised *Candida antarctica* lipase B (Novozym 435) was a gift from Novo Nordisk A/S (Bagsvaerd, Denmark). *Rhizomucor miehei* lipase (CHIRAZYME L-9, c-f, lyo.) was a gift from Boehringer–Mannheim (Mannheim, Germany). Enzymatic reactions were carried out in a New Brunswick Scientific Innova 4080 incubatory orbital shaker at 25°C. ^{1}H NMR spectra were recorded on a Varian Mercury 200BB (200 MHz) or a Bruker AC-E 200 (200 MHz) or a Bruker Avance DPX 400 (400 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Bruker AC-E 200 (50 MHz). Chemical shifts are reported in parts per million (ppm) relative to trimethylsilane (δ 0.00). Mass spectral data and accurate mass measurements were determined on a Finnigan MAT 95 mass-spectrometer. Melting points were determined on a Boetius apparatus and are uncorrected. Optical rotation values were measured with a Perkin–Elmer 241 digital polarimeter. Elemental analyses were determined on a Carlo-Erba elemental analyser. The reaction mixtures were analysed by HPLC on a 4.6×250 mm column packed with 5 μm Spherisorb ODS-2 (Phase Separations) with solvent system acetonitrile/water/acetic acid (60:40:0.1) as mobile phase at a flow rate of 1.0 mL/min using a Gynkotek 480 pump and Applied Biosystems 758A programmable absorbance detector at 254 nm. Determination of enantiomeric excesses of **4a** and **4b** was performed by analysis on an enantioselective column Chirex 3011, 4.6×250 mm, 5 μm (Phenomenex) using a Ginkotek 580A pump and an Applied Biosystems 759A absorbance detector at 254 nm. The eluent was methanol/dichloromethane (1:2) at a flow rate of 1.0 mL/min. Peak areas were determined electronically with the Chromeleon chromatography data system, Dionex Softron GmbH (Germering, Germany). The enantiomeric purity of (−)-(*R*)-**6b** was measured after coupling to (*R*)-(+)-α-methylbenzylamine on a reversed phase ODS-2 column.[14] The enantiomeric excess of (−)-(*R*)-**7** has been determined by ^{1}H NMR using a chiral shift reagent.[14] The enantiomeric excesses of the intermediate (−)-(*R*)-**5b** was assumed to be the same as for (−)-(*R*)-**4b**, (−)-(*S*)-**6b** and (−)-(*R*)-**7**.

4.2. Bis(2-cyanoethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 1

A solution of 2-(difluoromethoxy)benzaldehyde (10 g, 58 mmol), 2-cyanoethyl acetoacetate (18.2 g, 117 mmol) and 25% aqueous ammonium solution (10 mL, 130 mmol) in ethanol (50 mL) was stirred under reflux for 6 h. After cooling the mixture, the precipitate was filtered off and crystallised from ethanol to give yellow crystals of **1** (10 g, 38%): mp 138–139°C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.31 (s, 6H, 2,6-CH_3), 2.65 (t, 4H, J=6.8 Hz, 3,5-CH_2CN), 4.22 (t, 4H, J=6.8 Hz, 3,5-OCH_2-), 5.25 (s, 1H, 4-CH), 5.95 (br s, 1H, NH), 6.60 (t, 1H, $J_{H\text{-}F}$=78.0 Hz, $OCHF_2$), 7.00–7.19 (m, 3H, Ar-H), 7.37 (dd, 1H, J=2.2, 7.3 Hz, Ar-H); ^{13}C NMR ($CHCl_3$, 50 MHz): δ 17.95 (2×CH_2); 19.66 (2×CH_3); 35.33 (CH); 58.27 (2×CH_2); 101.94 (2×C); 116.97, (t, CH, J=274.2 Hz, $OCHF_2$); 117.34 (2×CN); 117.99 (CH), 125.28 (CH), 128.04 (CH), 131.57 (CH), 137.91

(C), 146.06 (2×C), 149.15 (C), 166.66 (2×C); MS *m/z* (rel. abund.): 445 (M+, 6); 375 (6), 347 (11), 303 (17), 302 (100), 278 (6), 277 (33), 249 (8), 205 (13), 196 (8); HRMS calcd for $C_{22}H_{21}F_2N_3O_5$ 445.1449, found 445.1442. Anal. calcd for $C_{22}H_{21}F_2N_3O_5$: C, 59.32; H, 4.75; N, 9.43; found: C, 59.33; H, 4.73; N, 9.40%.

4.3. 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylic acid, 2

To a stirred solution of **1** (2 g, 4.5 mmol) in ethanol (50 mL) was added a solution of KOH (0.55 g, 10 mmol) in ethanol (5 mL). After stirring the mixture at rt for 2 h the solvent was evaporated and the residue was dissolved in water. After cooling down the solution was adjusted to pH 4–5 by adding dilute aqueous HCl. The precipitate was filtered off and washed thoroughly with water to give **2** in pure form (1.11 g, 73%), mp 138–140°C; ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.19 (s, 6H, 2,6-CH_3), 5.08 (s, H, 4-CH), 6.78 (t, 1H, J=75.4 Hz, $OCHF_2$), 6.9–7.3 (m, 4H, Ar-H), 8.52 (br s, H, NH), 11.38 (br s, 2H, 3,5-COOH); ^{13}C NMR (DMSO-d_6, 200 MHz): δ 18.29 (2×CH_3); 34.90 (CH), 101.71 (2×C); 117.26 (t, CH, J=246.5 Hz, $OCHF_2$), 117.96 (CH), 125.23 (CH), 127.31 (CH), 131.15 (CH), 139.89 (C), 145.05 (2×C), 148.28 (C), 168.87 (2×C). Anal. calcd for $C_{16}H_{15}F_2NO_5$: C, 56.64; H, 4.46; N, 4.13; found: C, 56.32; H, 4.45; N, 4.20%.

4.4. Bis[(pivaloyloxy)methyl] 4-[2-(difluoromethoxy)-phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 3a

Compound **2** (3.66 g, 10.8 mmol) was dissolved in DMF (10 mL), after which K_2CO_3 (3.70 g, 26.9 mmol) was added. The mixture was stirred for 2 h at rt and pivaloyloxymethylchloride (3.74 mL, 25.92 mmol) was added and the resulting mixture was stirred for an additional 24 h. The reaction mixture was diluted with chloroform (100 mL), washed successively twice with water and twice with brine, dried and evaporated. The residue was triturated with methanol and crystallised from diluted methanol to give **3a** (3.5 g, 57%), mp 105–107°C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.06 (s, 18H, 3,5-C(CH_3)$_3$), 2.26 (s, 6H, 2,6-CH_3), 5.18 (s, H, 4-CH), 5.68 (s, 4H, 3,5-$COOCH_2O$), 6.04 (br s, H, NH), 6.51 (t, 1H, J=75.0 Hz, $OCHF_2$), 6.88–7.11 (m, 3H, Ar-H), 7.31 (dd, 1H; J=2.0, 7.3 Hz, Ar-H); ^{13}C ($CDCl_3$, 50 MHz): δ 19.79 (2×CH_3), 26.73 (6×CH_3), 36.33 (CH), 38.61 (2×C), 78.90 (2×CH_2), 101.62 (2×C), 116.79 (t, CH, J=255.5 Hz, $OCHF_2$), 117.54 (CH), 124.73 (CH), 127.78 (CH), 132.25 (CH), 136.94 (C), 146.13 (2×C), 150.22 (C), 162.84 (2×C), 177.14 (2×C). MS *m/z* (rel. abund.): 567 (M+, 11); 437 (8), 436 (33), 425 (22), 424 (100), 394 (11), 364 (7), 294 (9), 196 (17), 57 (14); HRMS calcd for $C_{28}H_{35}F_2NO_9$ 567.2288, found 567.2273. Anal. calcd for $C_{28}H_{35}F_2NO_9$: C, 59.25; H, 6.22; N, 2.47; found: C, 58.96; H, 6.22; N, 2.41%.

4.5. Bis[(isobutyryloxy)methyl] 4-[2-(difluoromethoxy)-phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate, 3b

To a stirred solution of **1** (2.00 g, 4.5 mmol) in ethanol (50 mL) was added a solution of KOH (0.55 g, 10 mmol) in ethanol (5 mL). After stirring the mixture at rt for 2 h the solvent was evaporated. The residue was diluted with dry DMF (5 mL), after which isobutyryloxymethyl chloride[19] (1.41 g, 10.35 mmol) was added. The reaction mixture was stirred for 18 h, then diluted with water and extracted with chloroform. The organic layer was washed successively twice with water and twice with brine, dried and evaporated. The resulting product was flash chromatographed on silica gel [3:1 petroleum ether (bp 40–60°C)/EtOAc] to give **3b** as a yellow oil (1.17 g, 48%): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.03 (d, 6H, J=6.8 Hz, 2×CH_3), 1.06 (d, 6H, J=6.8 Hz, 2×CH_3), 2.27 (s, 6H, 2,6-CH_3), 2.44 (septet, 2H, J=6.8 Hz, 3,5-CH(CH_3)$_2$), 5.18 (s, 1H, 4-CH), 5.69 (s, 4H, 3,5-$COOCH_2O$), 6.24 (br s, 1H, NH), 6.76 (t, 1H, $J_{H\text{-}F}$= 74.0 Hz, $OCHF_2$), 6.92–7.12 (m, 3H, Ar-H); 7.32 (dd, 1H, J=2.0, 7.3 Hz, Ar-H); ^{13}C ($CDCl_3$, 50 MHz): δ 18.53 (4×CH_3), 19.83 (2×CH_3), 33.61 (2×CH), 36.20 (CH), 78.70 (2×CH_2), 101.59 (2×C), 116.74 (t, CH, J=255.29 Hz, $OCHF_2$), 117.41 (CH), 124.60 (CH), 127.76 (CH), 132.21 (CH), 136.82 (C), 146.20 (2×C) 150.18 (C), 165.86 (2×C), 175.74 (2×C); MS *m/z* (rel. abund.): 539 (M+, 11), 422 (30), 397 (20), 396 (100), 322 (7), 297 (8), 296 (54), 294 (18), 196 (43), 71 (8), 43 (11). HRMS calcd for $C_{26}H_{31}NO_9F_2$ (M+) *m/z* 539.1967, found 539.1963.

4.6. (−)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-5-{[(pivaloyloxy)methoxy]carbonyl}-3-pyridinecarboxylic acid, 4a

A solution of **3a** (200 mg, 0.35 mmol) in acetonitrile (30 mL) was added to K_2HPO_4/KH_2PO_4 buffer (20 mM, pH 7.5, 500 mL) and heated to 45°C, after which Lipase AH (600 mg) was added. The resulting mixture was shaken at 350 rpm and 45°C for 5 days, then the pH of the solution was adjusted to 5.0 by adding 1 M aqueous HCl and extracted three times with ethyl acetate. The organic layers were dried and concentrated under reduced pressure. The residue was crystallised from methanol to give **4a** (140 mg, 87%), mp 175–177°C, $[\alpha]_D^{20}$ +1.3 (*c* 1.0, MeOH), e.e.=25%; ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.02 (s, 9H, C(CH_3)$_3$), 2.21 (s, 6H, 2,6-CH_3), 5.09 (s, H, 4-CH), 5.62 (s, 2H, $COOCH_2O$), 6.93 (t, 1H, J=75.0 Hz, $OCHF_2$), 6.93–7.25 (m, 4H, Ar-H), 8.92 (br s, H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz): 18.24 (CH_3), 18.65 (CH_3); 26.56 (3×CH_3), 35.12 (CH), 38.09 (C), 78.86 (CH_2), 99.21 (C), 102.43 (C), 117.11 (t, CH, J=254.74 Hz, $OCHF_2$), 117.61 (CH), 124.98 (CH), 127.62 (CH), 131.32 (CH), 138.55 (C), 144.68 (C), 148.20 (C), 148.84 (C), 165.66 (C), 168.75 (C), 176.40 (C); MS *m/z* (rel. abund.): 453 (M+, 2), 409 (23), 310 (18), 294 (25), 278 (55), 267 (16), 266 (100), 250 (14), 236 (35), 152 (71), 57 (20); HRMS calcd for $C_{22}H_{25}NO_7F_2$ (M+) *m/z* 453.1599, found 453.1592. Anal. calcd for $C_{22}H_{25}NO_7F_2$: C, 58.27; H, 5.56; N, 3.09; found: C, 57.95; H, 5.54; N, 3.00%.

4.7. (−)-(4*R*)-4-[2-(Difluoromethoxy)phenyl]-5-{[(isobutyryloxy)methoxy]carbonyl}-2,6-dimethyl-1,4-dihydro-3-pyridinecarboxylic acid, (−)-*R*-4b

To a solution of **3b** (0.539 g, 1 mmol) in water-saturated IPE (50 mL) was added *Candida rugosa* lipase (0.200 g) and the resulting mixture was shaken for 3.5 h at rt. After removal of the enzyme by filtration, the filtrate was concentrated under reduced pressure. The residue was flash chromatographed on silica gel with petroleum ether (bp 40–60°C)/chloroform/isopropyl alcohol (100:40:5→100:100:20) to give unreacted **3b** (0.125 g, 23%) and (−)-(*R*)-**4b** (0.240 g (55%), mp 149–151°C or 129–130°C (dec.), from ether–hexane; $[\alpha]_D^{20}$ +26.5 (*c* 1.0, $CHCl_3$), −24.0 (*c* 1.0 MeOH); e.e. ≥99%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.08 (d, 3H, J=6.8 Hz, CH_3), 1.11 (d, 3H, J=6.8 Hz, CH_3), 2.31 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.48 (septet, 1H, J=6.8 Hz, C*H*$(CH_3)_2$), 5.21 (s, 1H, 4-CH), 5.73 (ABq, 2H, $COOCH_2O$), 6.45 (dd, 1H, $J_{H\text{-}F}$=74.4, 76.0 Hz, $OCHF_2$), 7.00–7.16 (m, 3H, Ar-H), 7.37 (dd, 1H, J= 2.0, 7.6 Hz, Ar-H); ^{13}C ($CDCl_3$+CD_3OD, 50 MHz): δ 18.45 (2×CH_3), 18.69 (CH_3), 19.03 (CH_3), 33.73 (CH), 35.72 (CH), 78.77 (CH_2), 100.66 (C), 102.71 (C), 117.10 (t, CH, J=254.95 Hz, $OCHF_2$), 118.07 (CH), 124.98 (CH), 127.67 (CH), 131.88 (CH), 138.46 (C), 145.87 (C), 148.26 (C), 149.58 (C), 166.62 (C), 170.45 (C), 176.28 (C); MS *m*/*z* (rel. abund.): 439 (M^+, 2) 395 (17), 296 (12), 294 (19), 278 (28), 253 (9), 252 (64), 250 (11), 196 (13), 152 (100), 44 (18); HRMS calcd for $C_{21}H_{23}F_2NO_7$ 439.1443, found 439.1438. Anal. calcd for $C_{21}H_{23}NO_7F_2$: C, 57.40; H, 5.28; N, 3.19; found: C, 57.69; H, 5.12; N, 3.00%.

4.8. (−)-3-[(Isobutyryloxy)methyl] 5-methyl (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, (−)-*R*-5b

To a solution of (−)-(*R*)-**4b** (0.77 g, 1.75 mmol) in DMF (1 mL) was added K_2CO_3 (0.12 g, 1.75 mmol). The reaction mixture was stirred for 2 h at rt, after which MeI (0.218 mL, 3.5 mmol) was added and the mixture was stirred for another 2 h. The reaction mixture was diluted with water and extracted with chloroform. The organic layers were washed twice with water and brine, dried, and evaporated. The remaining residue was flash chromatographed on silica gel petroleum ether (bp 40–60°C)/chloroform/isopropyl alcohol (10:1:1) to give **5b** as a yellow oil (0.69 g, 1.52 mmol, 87%): $[\alpha]_D^{20}$ −17.6 (*c* 1.0, $CHCl_3$); e.e.≥99%; (lit.[14] 79% e.e., $[\alpha]_{20}^{D}$ −17.0 (*c* 1.0, $CHCl_3$)). The ^{1}H NMR spectrum was in accordance with the literature.[14] MS: *m*/*z* (rel. abund.) 453 (M+, 9), 352 (7), 336 (18), 311 (11), 310 (80), 308 (8), 294 (7), 211(11), 210 (100), 69 (11), 43 (7). HRMS calcd for $C_{22}H_{25}NO_7F_2$ 453.1599, found 453.1592.

4.9. (−)-(4*S*)-4-[2-(Difluoromethoxy)phenyl]-5-(methoxycarbonyl)-2,6-dimethyl-1,4-dihydro-3-pyridinecarboxylic acid, (−)-(*S*)-6b

This compound was prepared by the same method used in the literature.[14] The product was characterised giving: mp 78–79°C triturated from ether-hexane, e.e. ≥99% (lit.[14] mp 87–89°C, 79% e.e.); $[\alpha]_{20}^{D}$ −51.7 (*c* 1.0, $CHCl_3$) (lit.[14] $[\alpha]_{20}^{D}$ −46.9 (*c* 1.0, $CHCl_3$); the ^{1}H NMR was in accordance with the literature.[14]

4.10. (−)-3-Methyl 5-(2-propoxyethyl) (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, (−)-(*R*)-7

This compound was prepared by the same method used in the literature.[14] The oily product was characterised giving: e.e. ≥99%, $[\alpha]_{20}^{D}$ −19.7 (*c* 1.0, $CHCl_3$) (lit.[14] 88% e.e., $[\alpha]_{20}^{D}$ −17.0 (*c* 1.0, $CHCl_3$)). The ^{1}H NMR and mass spectral data were in accordance with the literature.[14]

Acknowledgements

Financial support by the Copernicus programme (ERB-CIPA-CT 94 0121), NATO (Linkage grant LST.CLG 974948), the PhD programme of Wageningen University and a PhD Grant of the Latvian Council of Science are gratefully acknowledged. We are indebted to Mr. A. van Veldhuizen for recording the NMR spectra, to Mr. C. J. Teunis and Mr. H. Jongejan for the mass spectral analyses and to Mr. E. J. C. van der Klift and Emma Sarule for the elemental analyses.

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