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Enzymatic synthesis of enantio- and diastereomerically enriched *syn*-3-nitro-2-pentanol

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Abstract

The enzymatic resolution of a commercial mixture of nitropentanol isomers was achieved. The *syn* isomer could be obtained in high de and ee in either the (2*S*,3*S*) or (2*R*,3*R*) configuration by combining an enantioselective enzymatic acylation or deacylation with a stereoselective elimination of the minor *anti* isomer.

1. Introduction

SCH56592 (**1**) is an azole anti-fungal with superior activity against systemic *Candida* and *Aspergillus* infections, and is currently in phase II clinical trials for treating both normal and immunocompromised patients. Unlike related azole anti-fungals (e.g. itraconazole) which contain a central dioxolane ring, SCH56592 contains a trisubstituted tetrahydrofuran and a hydroxylated side chain. The absolute configurations of the stereocenters on both the central tetrahydrofuran ring and on the side chain are crucial for the enhanced anti-fungal activity of SCH56592.[1]

Continuing our efforts towards the chemoenzymatic synthesis of key chiral intermediates of SCH56592,[2] we required a source of (2*S*,3*S*)-3-nitro-2-pentanol (**2**). Reduction of the nitro group to give the aminoalcohol, followed by diaziridine formation, hydrolysis and formylation, would provide formylhydrazone **5**, a key intermediate for construction of the triazolone ring and the hydroxylated side chain of SCH56592 (Scheme 1).

Nitroalcohols are versatile building blocks which are easily prepared via the classic Henry reaction.[3] The nitro group can be readily reduced and the resulting 1,2-aminoalcohols, when enantiopure, are

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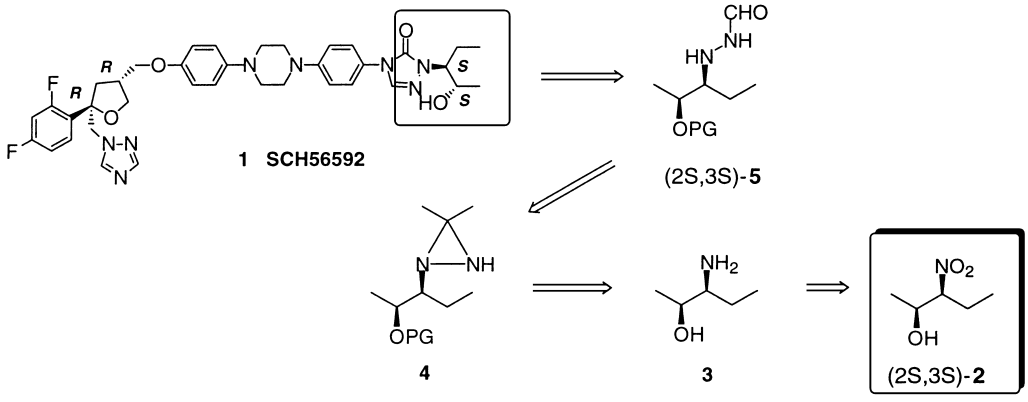


Scheme 1.

important chiral intermediates for many pharmaceuticals and natural products, and are also widely used in the preparation of chiral auxiliaries.[4]

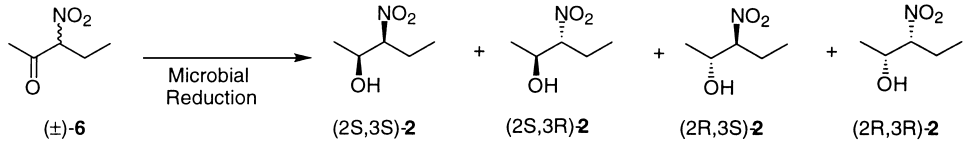
Chiral nitroalcohols have been prepared previously in enantiopure form by microbial reduction of nitroketones,[5] enzymatic resolution of nitroalcohols,[5c,6] enzymatic desymmetrization of nitrodiacetates[7] and by enantio- and diastereoselective aldol reactions.[8]

In this report we outline our experience in the synthesis of (2*S*,3*S*)-3-nitro-2-pentanol (**2**), either by microbial reduction of 3-nitro-2-pentanone (**6**) or by enzymatic resolution of (±)-**2**. We also report a convenient method for the preparation of enantiopure *syn*-nitropentanol by the selective elimination of acylated *syn*:*anti* mixtures.

2. Results

2.1. Microbial reduction of 3-nitro-2-pentanone (6)

While the reduction of β- and γ-nitroketones has been performed successfully using baker's yeast,[5] reductions of α-nitroketones have been less productive.[6b] A major attraction for investigating the enantioselective microbial reduction of (±)-3-nitro-2-pentanone (**6**) was the possibility that the unreacted enantiomer might undergo racemization under the reaction conditions, resulting in a high-yield dynamic resolution (Scheme 2). Therefore, a screen for the reduction of (±)-**6** (0.5–1.0 g/L) to (2*S*,3*S*)-3-nitro-2-pentanol (**2**) was conducted. Among the 1082 cultures of yeast, filamentous fungi and bacteria surveyed, over half appeared to either completely degrade the substrate or accumulate unknown products. Most of the remaining cultures demonstrated poor diastereoselectivity and many produced all four possible isomers. However, 50 cultures produced **2** with high ee but with variable de. Of these, *Hansenula subpelliculosa* (ATCC 16766) predominantly produced the desired (2*S*,3*S*)-**2** and was chosen for process optimization.



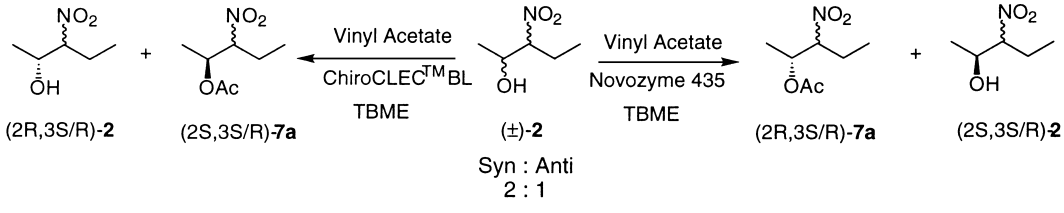
Scheme 2.

Bioconversion of **6** (2 g/L) using *H. subpelliculosa* revealed that most of the substrate was consumed following 6 h of incubation to give nitropentanol **2** along with nitropropane. Retro-Henry reversion of **2** to nitropropane occurred in the absence of cells at pH>3. While this was significantly reduced in the presence of growing cells by addition of glucose, culture productivity was subject to repressive regulation by glucose. Therefore, bioconversions were conducted employing resting cells resuspended in phosphate–citrate buffer (pH 3) supplemented with glucose (3%). Conversion yields as high as 80–90% at 5 g/L of **6** were achieved within 2 h using 10X cell concentrates. However, reaction selectivity decreased with increased substrate concentration, with both low or high density cell cultures. Elevated substrate concentrations, especially in the presence of low cell density, resulted in increasing yields of the undesired 2*R* product diastereomers, suggesting that 3-nitro-2-pentanone (**6**) was likely reduced by more than one enzyme. In order to minimize this activity, low substrate concentrations (<1 g/L) and high cell densities (20X concentrate) were necessary but not economical. Consequently, no further development of this reaction was pursued.

*2.2. Enzymatic kinetic resolution of (±)-3-nitro-2-pentanol (**2**)*

2.2.1. Screen results

Commercially available (±)-3-nitro-2-pentanol (**2**) exists as a ~2:1 *syn*:*anti* mixture.[9] A screen of 210 enzyme preparations was undertaken to identify catalysts capable of the diastereoselective and enantioselective acylation of **2**. Twenty enzyme preparations were reactive under the conditions of the screen (vinyl acetate in TBME), all of them showing a slight preference for acylation of the *syn* isomer at low conversion. With one exception, all the enzymes preferentially acetylated the 2*R* stereogenic center. Two enzymes, Novozyme 435 and ChiroCLEC™ BL,[10] showing opposite enantioselectivity (*R* and *S* selective, respectively[11]), were chosen for further investigation (Scheme 3).



Scheme 3.

*2.2.2. Enzymatic kinetic resolution of (±)-3-nitro-2-pentanol (**2**) with ChiroCLEC™ BL*

Since ChiroCLEC™ BL catalyzed the acylation of the desired (2*S*,3*S*/*R*)-isomers, the reaction is a less convenient direct resolution, with an extra step required to convert the product ester back to the desired alcohol. Deacylation of (2*S*,3*S*/*R*)-**7a** under basic conditions was expected to be a problem because of the facile retro-Henry reaction of the nitropentanol **2**.

Because ChiroCLEC™ BL showed unique selectivity, 21 other hydrolytic enzyme preparations (derived from *B. licheniformis* or *Bacillus* sp.) were examined for their ability to acetylate (±)-**2** using vinyl acetate in TBME. Again, only ChiroCLEC™ BL showed any activity under these conditions, providing (2*S*,3*S*)-**7a** with high ee but with poor diastereoselectivity (*syn*-**7a**, 94.3% ee; conversion, 49%; E[12] >100). Interestingly, in a companion screen of 24 *Bacillus* sp. hydrolases, ChiroCLEC™ BL showed no reactivity for the hydrolysis of (±)-nitroacetate **7a** in phosphate buffer (pH 7) (both the dry form, for reaction in organic solvents, and the wet form, for hydrolysis reactions, were examined). The only two enzymes which did show activity, Interspex Protease/Esterase (*B. licheniformis*) and Fluka Esterase

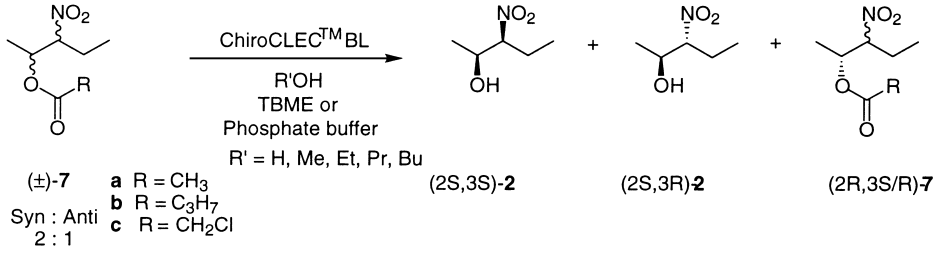
(*Bacillus* sp.), both showed a preference for hydrolysis of the (2*R*,3*S*/*R*)-isomers, albeit with poor enantio- and diastereoselectivity.

The ChiroCLEC™ BL catalyzed acetylation worked well in a number of common solvents, with the best results being observed in *t*-amyl alcohol, TBME and THF. In all cases the enantioselectivity was good, with the *syn*-(2*S*,3*S*)-**7a** being formed in 93–98% ee; the *anti*-(2*S*,3*R*)-**7a** was formed less selectively (89–97% ee). No attempt was made to adjust the water content of the reactions even though the performance of CLECs are reported to be susceptible to water activity.[13]

The ChiroCLEC™ BL catalyzed acylation showed a marked dependence on the nature of the acetylating agent, faster rates being observed with vinyl acetate than with isopropenyl acetate or trifluoroethyl acetate. Poor results were observed in neat alkyl acetates and also, surprisingly, with acetic anhydride. When tested again under similar conditions acetic anhydride showed poor reactivity and selectivity while propionic, butyric and isobutyric anhydrides all showed good reactivity; isobutyric anhydride also displayed good enantioselectivity in the acylation of *syn*-nitroalcohol (ee_s >95%, ee_p 91.6%, c 52%, *E*=105). Reaction with chloroacetic anhydride occurred rapidly, but produced racemic product.

Because enzymes show the same enantioselectivity for acylation and deacylation reactions, CLEC catalyzed alcoholysis of nitropentanol esters appeared attractive since (i) it would produce the alcohol with the desired 2*S* configuration and (ii) it was possible to prepare *syn*-esters chemically from *syn*/*anti* mixtures (vide infra).

In a comparison of the CLEC catalyzed deacylation/hydrolysis of three nitroesters **7a**–**c** (Scheme 4), the best results were observed for the deacylation of the propionate **7b** and the chloroacetate **7c** with *n*PrOH and *n*BuOH; MeOH performed poorly (~16% conversion for **7c**) and no reaction was observed with the acetate **7a** under all conditions.



Scheme 4.

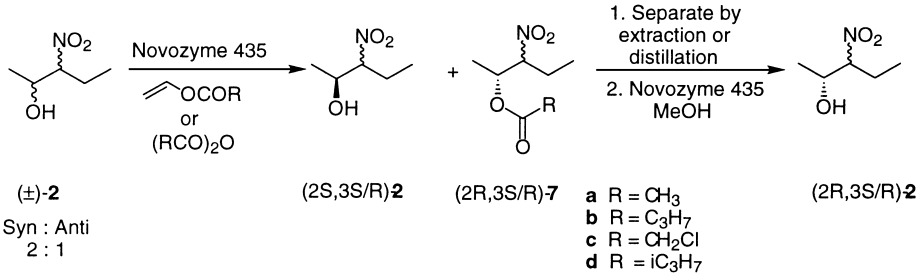
One major problem with the ChiroCLEC™ BL catalyzed kinetic resolution of nitropentanol, under both acylation and deacylation conditions, was a lack of reproducibility, particularly on scale up. The deacylation of the chloroacetate **7c** with propanol in heptane worked well on 100 mg scale (50% conversion after 24 h using 25% enzyme loading). When scaled to 1 g (using 10% enzyme loading) the reaction stalled at only 34% conversion after 45 h.

Because of the apparent lack of reproducibility and the relative expense of the catalyst, the alternative Novozyme 435 catalyzed resolution was favored.

*2.2.3. Enzymatic kinetic resolution of (±)-3-nitro-2-pentanol (**2**) using Novozyme 435*

To establish the enantiopreference of Novozyme 435, initial experiments concentrated on an enzymatic acylation/separation/enzymatic deacylation sequence, which allowed access to the undesired (2*R*,3*S*/*R*)-**2** (Scheme 5). It has now been established that, as for other secondary alcohols,[14] Novozyme 435 acylates the undesired (2*R*,3*S*/*R*)-isomers of **2**,[15] permitting a more convenient subtractive resolution.

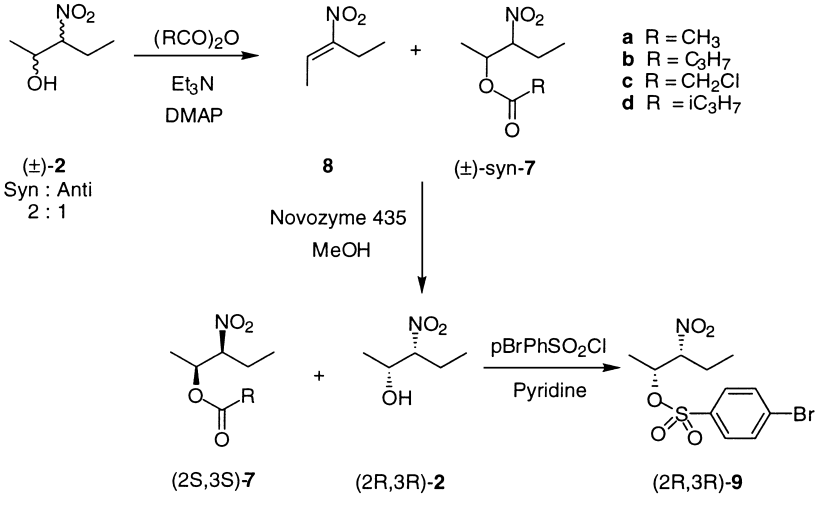
High enantioselectivity was achieved using a number of vinyl esters as acylating agents (Scheme 5). Chloroacetate formation was particularly convenient since (2*R*,3*S*/*R*)-**7c** might be easily isolated by



Scheme 5.

distillation from the initial enzymatic resolution, and the subsequent enzymatic deacylation was rapid. Enzymatic deacylation of the acetate (2*R*,3*S*/*R*)-**7a** and particularly the isobutyrate (2*R*,3*S*/*R*)-**7d** was often sluggish when using the enzyme recovered from the initial resolution step.

When investigating the alternative chemical acylation/enzymatic deacylation procedure (Scheme 6 and Fig. 1), a significant observation was made. During chemical acylation of (±)-**2** (anhydride/Et_3N) a major byproduct was formed which was identified as (*E*)-3-nitro-2-pentene (**8**). More importantly the nitropentene was formed more rapidly from the undesired *anti* diastereomer.[16] Controlling the acylation conditions resulted in complete elimination of the undesired *anti*-nitropentanol isomer to give nitropentene **8**. This could be easily removed by distillation, leaving diastereomerically pure *syn*-nitroalcohol to be resolved enzymatically in the next step. Depending on the order of the reactions (chemical acylation/elimination/enzymatic acylation/enzymatic deacylation), the choice of enzyme (ChiroCLEC™ BL or Novozyme 435) and the workup procedure, access to optically enriched (2*S*,3*S*)- or (2*R*,3*R*)-**2** or the corresponding esters **7** was possible.



Scheme 6.

Enzymatic methanolysis of *syn*-nitroacetate (±)-**7a**, isolated after elimination of the *anti* isomer, was sometimes slow and the results erratic. The possibility that nitropentene **8** was inhibiting the enzyme was suggested by several reactions using crude reaction mixtures. For example, after chemical acetylation, elimination and workup, a mixture of *syn*-(±)-**7a** and **8** showed no reaction when treated with Novozyme 435 and MeOH. Furthermore, the enzyme recovered from this failed methanolysis showed no further activity in the methanolysis of purified (±)-**7a**, nor in the acetylation of (±)-nitroalcohol **2**. Nitropentene is a powerful Michael acceptor and might be expected to irreversibly inactivate hydrolytic enzymes

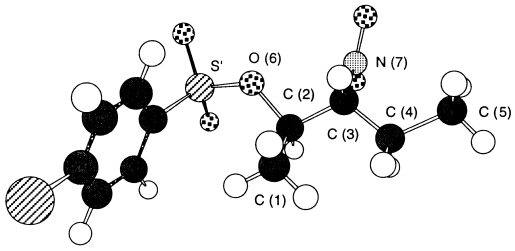
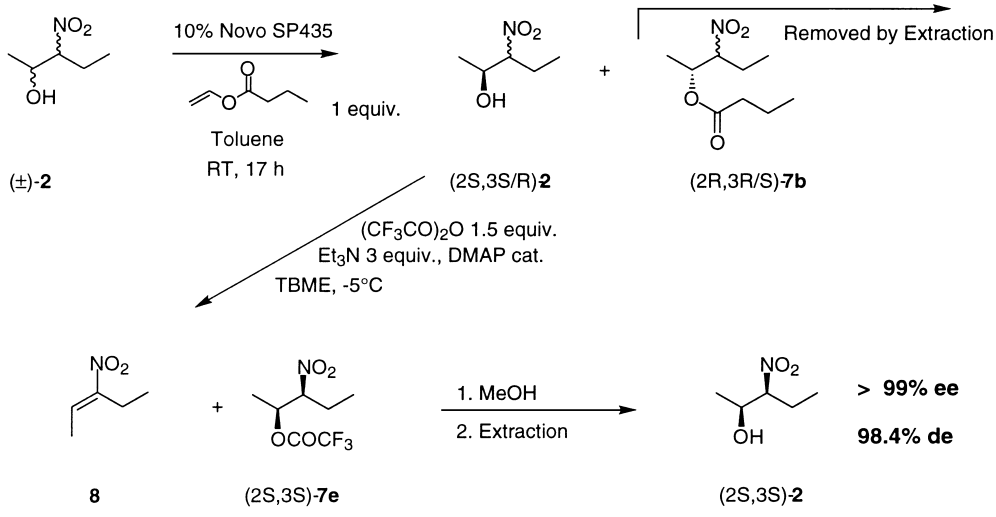


Figure 1. ChemDraw representation of the X-ray crystal structure of *syn*-(2*R*,3*R*)-brosylate **9**

by alkylation of the serine hydroxyl at the active site. Similarly, the propanolysis of (±)-*syn*-**7c** using ChiroCLEC™ BL in the presence and absence of nitropentene **8** showed essentially no reaction (<5%), while the deacylation of (2*S*,3*S*)-**7c** and *syn*/*anti*-(±)-**7c** ran as expected. This suggested that the enzyme might still be inactivated by traces of nitropentene **8** remaining after purification of the *syn*-ester or even by nitropentene adventitiously formed during the deacylation reaction.

Accordingly, the best course of action was to carry out the enzymatic acylation first and isolate the *syn*/*anti*-(2*S*,3*S*/*R*)-**7**, then chemically acylate and eliminate using a second labile acylating agent. Following this the pure *syn*-alcohol (2*S*,3*S*)-**7** could be separated cleanly from nitropentene **8**. For this purpose the use of trifluoroacetic anhydride was especially convenient (Scheme 7).



Scheme 7.

Using this enzymatic acylation/chemical acylation/elimination method, (2*S*,3*S*)-**2** could be obtained in >99% ee and 98% de. The ~23% isolated yield was quite respectable since the theoretical yield was only 30% (kinetic resolution of a 2:1 *syn*:*anti* mixture).

3. Experimental

3.1. Material and methods

3.1.1. Analytical methods

Chiral GC was performed on a Shimadzu GC-14A equipped with a Chiraldex B-TA capillary column (Astec, Whippany, NJ) (40 m×0.25 mm×0.25 mm) [run conditions 100°C for 11 min; 5°/min to 150°C

for 2 min; retention times (min): acetates **7a**, (2*R*,3*S*), 7.7, (2*S*,3*R*), 8.1, (2*S*,3*S*), 9.1, (2*R*,3*R*), 9.5; alcohol **2**, (2*S*,3*R*), 14.4, (2*R*,3*S*), 15.3, (2*R*,3*R*), 17.5, (2*R*,3*R*), 17.5; chloroacetate **7d**, (2*R*,3*S*), 20.0, (2*S*,3*R*), 20.4, (2*R*,3*R*), 21.2, (2*S*,3*S*), 21.4; butyrate **7b**, 15.1, 16.2 (*anti*), 17.5 (*syn*, does not resolve)]. Optical rotations were determined on a Perkin–Elmer 243 B Polarimeter. Vinyl butyrate and vinyl chloroacetate were obtained from TCI USA (Portland, OR) and vinyl acetate 3-nitro-2-pentanol from Aldrich (Milwaukee, WI). All chemicals were used as received. Flash chromatography was carried out with Sorbisil C60 (40/60A) (Fisher Scientific). Novozyme 435 was purchased from Novo Nordisk (Danbury, CT) and ChiroCLEC™ BL from Altus Biologics Inc. (Cambridge, MA) and were used as received.

3.2. syn-(2R,3R)-3-Nitro-2-pentyl chloroacetate (7c) (enzymatic deacylation with ChiroCLEC™ BL)

A mixture of *syn*-(±)-**7c** (1.0 g, 4.8 mmol), *n*PrOH (3.6 mL, 47.7 mmol) and ChiroCLEC™ BL (0.1 g) in heptane (100 mL) was shaken at 40°C at 200 rpm. After a fast initial rate (20% conversion in 1.5 h) the reaction slowed considerably (34% conversion after 41 h). At this stage the reaction mixture showed ee_s 45.4%, ee_p 95.0%, c 32.3 and E=61. A further portion of enzyme was added (150 mg) and shaking continued for a total of 64 h (ee_s 98.2%, ee_p 88.0%, c 52.7%, E=73). The reaction mixture was filtered and the solvent evaporated. The residue was dissolved in toluene (10 mL) and extracted with 50% MeOH/water (5×10 mL). The toluene layer was dried ($MgSO_4$), filtered and evaporated to obtain (2*R*,3*R*)-**7c** (0.40 g, 40.3%; 98.1% ee): $[\alpha]_D^{25}$=+13.87 (c 1.810, EtOH); IR (Nujol): 1750 (C=O), 1555, 1460, 1311, 1188, 1093, 950, 871, 812, 721, 582 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.98 (t, 3H, J=7.4 Hz), 1.34 (d, 3H, J=6.4 Hz), 1.86 (ddq, 1H, J=10.0, 7.2, 4.0 Hz), 1.93 (ddq, 1H, J=10.0, 7.2, 7.2 Hz), 4.00 (s, 3H), 4.51 (ddd, 1H, J=10.1, 8.8, 3.6 Hz), 5.35 (dq, 1H, J=8.4, 6.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.06, 16.61, 23.49, 40.74, 71.98, 92.09, 165.94. The MeOH/water extracts were concentrated, extracted with EtOAc and the EtOAc extract dried ($MgSO_4$), filtered and evaporated to yield *syn*-(2*S*,3*S*)-3-nitro-2-pentanol (**2**) (0.18 g, 27.8%; 88.0% ee).

3.3. (2R,3R/S)-3-Nitro-2-pentanol (2) (enzymatic acylation/enzymatic deacylation)

A mixture of (±)-3-nitro-2-pentanol (**2**) (30 mL, 0.24 mol), vinyl acetate (13 mL, 0.12 mol) and Novozyme 435 (1.0 g) was shaken in toluene (200 mL) at room temperature at 175 rpm. After 23 h the reaction was filtered and the enzyme beads washed with toluene. The filtrate was extracted with 50% MeOH/water (8×200 mL), by which time GC indicated that <1% of the unreacted *syn*-alcohol was present. The toluene solution was then washed with satd NaCl (100 mL), dried ($MgSO_4$) and filtered.

The filtrate, containing (2*R*,3*R*/*S*)-**7a**, was treated with MeOH (25 mL, 0.62 mol) and Novozyme 435 (1 g fresh enzyme and 1 g recovered enzyme) and shaken at room temperature at 175 rpm. After 120 h the *syn*-acetate was completely deacylated (~7% *anti*-acetate remained). The reaction mixture was filtered, the enzyme beads washed with toluene and the combined filtrate evaporated. The residue (9.8 g) was distilled under reduced pressure to yield (2*R*,3*R*/*S*)-**2** (7.5 g, 23.3%) (*syn*-**2**, 99.1% ee; *anti*-**2**, 92.2% ee; 79.5% de) (product contained ~10% *anti*-**7a**): $[\alpha]_D^{25}$=+2.54 (c 1.024, EtOH); IR (neat on KBr): 3500 (b), 2975, 2941, 2884, 1552, 1460, 1376, 1130, 1107, 809 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.98 (t, 3H, J=7.4 Hz), 1.34 (d, 3H, J=6.4 Hz), 1.86 (ddq, 1H, J=10.0, 7.2, 4.0 Hz), 1.93 (ddq, 1H, J=10.0, 7.2, 7.2 Hz), 4.00 (s, 3H), 4.51 (ddd, 1H, J=10.1, 8.8, 3.6 Hz), 5.35 (dq, 1H, J=8.4, 6.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.37, 11.71 (*anti*), 20.24 (*anti*), 20.92, 24.01 (*anti*), 24.97, 69.47, 70.65 (*anti*), 95.74 (*anti*), 96.86.

3.4. (2R,3R)-3-Nitro-2-pentanol (2) (chemical acylation/elimination/enzymatic deacylation)

Acetic anhydride (50 mL, 0.53 mol) was added dropwise to a solution of 3-nitro-2-pentanol (**2**) (50 mL, 0.40 mol) and triethylamine (85 mL, 0.61 mol) in TBME (200 mL) at 0°C, followed by dimethylaminopyridine (0.25 g, 0.2 mmol). After 45 min at 0°C, the reaction was warmed to room temperature and monitored closely by GC. After 4 h the *anti*-nitroacetate **7a** was completely consumed. The reaction mixture was quenched with water (100 mL) and washed with 1.2 M HCl (4×100 mL), water (100 mL), satd $NaHCO_3$ (2×100 mL), water (100 mL) and satd NaCl (100 mL), then dried ($MgSO_4$), filtered and evaporated. The residue was distilled under reduced pressure collecting the *syn*-nitroacetate **7a** (100–107°C/24 mmHg) (27.5 g, 38.9%).

A mixture of *syn*-(±)-**7a** (27 g, 0.15 mol), MeOH (30 mL, 0.8 mol) and Novozyme 435 (5.4 g) was shaken in toluene (200 mL) at 40°C at 200 rpm. After 39 h, chiral GC indicated the following: ee_s, 93.9%; ee_p, >99.0%; c, 48.6%. The reaction mixture was filtered after 111 h. The filtrate was extracted with 50% MeOH/water (10×100 mL). The toluene layer was dried (MgSO4), filtered and evaporated to obtain (2*S*,3*S*)-**7a** (10.8 g), which was purified by column chromatography (8.54 g, 31.6% yield based on (±)-acetate; 96.4% ee): $[\alpha]_D^{25}$=−22.0 (c 1.391, EtOH); IR (neat on KBr): 3447 (b), 2981, 2943, 2885, 1747 (C=O), 1555, 1458, 1376, 1233, 1097 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.00 (t, 3H, J=7.4 Hz), 1.31 (d, 3H, J=6.5 Hz), 1.81–2.02 (m, 2H), 2.03 (s, 3H), 4.50 (ddd, 1H, J=10.8, 8.5, 3.9 Hz), 5.29 (dq, 1H, J=8.6, 6.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 9.95, 16.60, 20.89, 23.33, 70.07, 92.56, 169.69.

The MeOH/water extracts were concentrated to remove MeOH, saturated with NaCl (50 g) and extracted with EtOAc (100 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated, and the residue purified by column chromatography to obtain (2*R*,3*R*)-**2** (7.41 g, 33.7% yield based on (±)-acetate **7a**; >99.0% ee): $[\alpha]_D^{25}$=+3.24 (c 1.111, EtOH); IR (neat on KBr): 3439 (b), 2980, 2941, 2884, 1553, 1465, 1378, 1265, 1131, 1108, 810 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.98 (t, 3H, J=7.4 Hz), 1.27 (d, 3H, J=6.4 Hz), 1.79–1.87 (m, 1H), 1.91–2.03 (m, 1H), 3.03 (bs, 1H, D_2O exch.), 4.14 (quin, 1H, J=7.5, 6.5 Hz), 4.34 (ddd, 1H, J=7.6, 7.6, 4.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.13, 19.70, 23.76, 68.43, 95.88.

3.5. (2S,3S)-3-Nitro-2-pentanol (2) (enzymatic acylation/chemical acylation/elimination)

Novozyme 435 (3 g) was added to a solution of (±)-**2** (30.1 g, 0.23 mol) and vinyl butyrate (25.2 g, 0.22 mol) and the mixture stirred at room temperature for 17 h. The reaction was filtered and the filtrate extracted with 50% MeOH/water (7×100 mL). The aqueous MeOH extracts were back-extracted with heptane (100 mL), then concentrated to remove the MeOH. NaCl (60 g) was added to the residue which was then extracted with EtOAc (2×100 mL). The organic layer was dried, filtered and evaporated to obtain nitroalcohol **2** (14.9 g; 49.4%) (>99.0% ee, 37.1% de; ~2% butyrate **7b** present).

A solution of the nitroalcohol **2** (14.9 g, 0.11 mol), triethylamine (46.7 mL, 0.34 mol) and dimethylaminopyridine (74 mg, 0.5%) in TBME (200 mL) was stirred under N_2 and cooled to −10°C. Trifluoroacetic anhydride (23.8 mL, 0.17 mol) was added slowly dropwise, maintaining the internal temperature of the reaction at <−5°C. After 3 h, GC indicated complete consumption of the *anti* isomer. The reaction mixture was quenched with water (100 mL), washed with 1.2 M HCl (2×100 mL), water (100 mL), satd $NaHCO_3$ (100 mL), water (100 mL) and satd NaCl (50 mL), dried ($MgSO_4$), filtered and evaporated. The residue, containing *syn*-trifluoroacetate **7e**, *syn*-nitroalcohol **2** and nitropentene **8**, was dissolved in MeOH (50 mL) and stirred overnight. The mixture was then diluted with MeOH (50 mL) and water (100 mL) and extracted with heptane (3×100 mL) to remove nitropentene. The aqueous layer was then

concentrated and saturated with NaCl, and extracted with EtOAc (2×50 mL). The EtOAc layer was dried ($MgSO_4$), filtered and evaporated to give (2*S*,3*S*)-**2** (8.2 g, crude 27%; >99.0% ee and 98.4% de).

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10. Novozyme 435 (Novo SP 435 from Novo Nordisk) is a lipase (lipase type B) whose gene coding has been transferred from a selected strain of *Candida antarctica* to a host organism, *Aspergillus oryzae*. The enzyme produced by the host organism is immobilized on a macroporous acrylic resin. ChiroCLEC™ BL (Altus Biologics, Inc.) is a cross-linked microcrystal of the serine endoprotease from *Bacillus licheniformis*. The dry powder form for reactions in organic solvents was used.
11. The absolute configurations were established as follows. Pure nitroacetate **7a** (*syn*/*anti* mixture), from the Novozyme 435 catalyzed acetylation of **2**, was reduced (Bu_3SnH/AIBN) and compared on chiral GC with the pentyl acetate prepared from commercial (*R*)-2-pentanol, establishing the reactive isomer as 2*R* (*syn*/*anti*). Similarly, pure *anti*-nitroacetate **7a** was reduced to (*R*)-pentyl acetate. This established the reactive isomers in the enzymatic acetylation as (2*R*,3*R*)-*syn* and (2*R*,3*S*)-*anti*. In addition, Novozyme 435 catalyzed deacylation of pure (±)-*syn*-**7a** provided (2*R*,3*R*)-**2** whose structure was confirmed by X-ray crystallography of the corresponding brosylate **9** (Scheme 6). The assignments were also confirmed by examination of the corresponding Mosher esters using the model proposed by Kakisawa: Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc*. **1991**, *113*, 4092–4096.
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