Enantiodivergent Chemoenzymatic Synthesis of (*R*)- and (*S*)-*β*-Proline in High Optical Purity

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A number of studies were recently directed toward elaboration of new synthetic approaches to the enantiomerically pure forms of nonproteinogenic amino acids.[1] For instance, peptide structures involving the pyrrolidine moiety have received considerable attention over the past few years,[2] and recent reports have focused on the use of such pyrrolidine analogues to prepare biologically active compounds.[2,3]

Also, proline analogues have been shown to be important tools for understanding the structure−activity relationship between receptors and natural amino acids or peptides.[4] Although several synthetic approaches to racemic 3-substituted pyrrolidines have been reported,[5] methods for the preparation of these compounds in enantiomerically pure form are scarcely found in the literature.[3a,6] The dextrorotatory antipode of *β*-proline, which was proven to be of (*S*) absolute configuration, was prepared in low yield (13% from a precursor which synthesis was not described) *via* fractional crystallization of a cinchonine derivative. Similarly, the (−)-antipode could only be obtained with a very low degree of optical purity following this procedure.[7] We have been ourselves interested in developing new and efficient chemoenzymatic methodologies for the synthesis of various valuable enantiomericaly pure chirons.[8,9] In this context, we present here our synthesis of both enantiomers of *β*-proline **1** (3-carboxypyrrolidine).[10]

Results and Discussion

Our approach was based on the use of an enantiodivergent strategy, starting from one single enantiomer of the enantiopure lactone **2**, followed by regioselective synthetic modifications to obtain both enantiomers of *β*-proline **1** (Scheme 1). Therefore, our first goal was to achieve the synthesis of this desired key-lactone **2**, in either enantiopure form. It was very tempting to prepare this chiral building block using a biocatalyzed Baeyer−Villiger oxidation of prochiral 3-[(benzyloxy)methyl]cyclobutanone (**3**).[11] We first explored the possibility to use previously described bacterial strains, i.e. *Acinetobacter calcoaceticus* NCIMB 9871, *Acinetobacter* TD 63, and *Pseudomonas putida* NCIMB 10007. However, none of these strains led to satisfactory results; the ees obtained were rather low in all cases.[12] A further screening of over 80 strains, including bacteria and fungi, led us to select the fungus *Cunninghamella echinulata* NRRL 3655. Using a resting-cell suspension of this fungus, oxidation of cyclobutanone **3** led to the stereoselective incorporation of an oxygen atom into one single enantiotopic C−C bond adjacent to the carbonyl moiety, thus affording the (*R*)-(−)-*γ*-butyrolactone **2** with a good yield and very high enantiomeric purity (60% yield, ee ≥ 97%).[13] It must be emphasized that this reaction has not been optimized at that stage and could, at our sense, lead to even higher yields if necessary.

As outlined in Scheme 1, the direct transformation of the lactone ring of (*R*)-(−)-**2** into a diol would open the way to the (*S*)-(+)-*β*-proline **1** enantiomer, whereas functionalization of the lateral chain of (*R*)-(−)-**2** into a primary amine should allow access to (*R*)-(−)-*β*-proline **1**. These synthetic pathways do not involve the asymmetric carbon of starting (*R*)-(−)-**2**, thus avoiding any possible loss of enantiomeric purity. All the reaction conditions were set up first starting from the racemic lactone (±)-**2**, which was easily available upon chemical Baeyer−Villiger oxidation of **3** using *m*-CPBA.

Synthesis of (*S*)-(+)-**1** was achieved as detailed in Scheme 2. Indeed, our first project was to transform directly the chiral lactone (*R*)-(−)-**2** into the corresponding lactam. However, all our attempts, carried out following various literature procedures,[14] failed in our hands and led either to no reaction at all or to complex mixtures of products with low isolated yields of the desired lactam.

The following alternative approach proved, however, to be successful. Chiral lactone (*R*)-(−)-**2** was reduced by lithium aluminum hydride into the diol (*S*)-(−)-**4**. Reaction of (*S*)-(−)-**4** with mesyl chloride gave, instantaneously, the dimesylate (*R*)-(−)-**5**, which was further reacted with benzylamine to afford the dibenzylpyrrolidine (*S*)-(+)-**6**. Hydrogenolysis (over Pd−C as a catalyst) using methanol as a solvent yielded the monodeprotected

(1) (a) Galeazzi, R.; Mobbili, G.; Orena, M. *Tetrahedron* **1996**, *52*, 1069, and references therein.
(2) Mulzer, J.; Meier, A.; Buschmann, J.; Luger, P. *J. Org. Chem.* **1996**, *61*, 566, and references therein.
(3) (a) Gmeiner, P.; Orecher, F.; Thomas, C.; Weber, K. *Tetrahedron Lett.* **1995**, 381. (b) Frolund, B.; Kristiansen, U.; Brehm, L.; Hansen, A. B.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1995**, *38*, 3287. (c) Cardillo, B.; Galeazzi, R.; Mobbili, G.; Orena, M.; Rossetti, M. *Heterocycles* **1994**, *38*, 2883. (d) Nielsen, L.; Brehm, L.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1990**, *33*, 71. (e) Anderson, G. T.; Alexander, M. D.; Taylor, S. C.; Smithrud, D. B.; Benkovic, S. J.; Weinreb, S. M. *J. Org. Chem.* **1996**, *61*, 125. (f) Horwell, D. C.; Howson, W; Naylor, D.; Willems, M. G. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1445. (g) Ramuzon, P. *Tetrahedron* **1996**, *52*, 13803.
(4) Sharma, R.; Lubell, W. D. *J. Org. Chem.* **1996**, *61*, 202.
(5) (a) Denmark, S. E.; Marcin, L. R. *J. Org. Chem.* **1993**, *58*, 3857. (b) Hagen, T. J. *Synlett* **1990**, 63.
(6) (a) Westrum, L. J.; Meyers, A. I. *Tetrahedron Lett.* **1994**, 973. (b) Meyers, A. I.; Snyder, J. *J. Org. Chem.* **1993**, *58*, 36.
(7) Fles, D.; Ghyczy, T. *Croat. Chem. Acta* **1964**, *36*, 27.
(8) (a) Viazzo, P.; Alphand, V.; Furstoss, R. *Tetrahedron Lett.* **1996**, 4519. (b) Archelas, A.; Delbecque, J.-P.; Furstoss, R. *Tetrahedron: Asymmetry* **1993**, 2445.
(9) (a) Lebreton, J.; Alphand, V.; Furstoss, R. *Tetrahedron* **1997**, *53*, 145. (b) Lebreton, J.; Alphand, V.; Furstoss, R. *Tetrahedron Lett.* **1995**, 1011. (c) Alphand, V; Furstoss, R.; Pedragosa-Moreau, S.; Roberts, S. M.; Willetts, A. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1867.
(10) (a) Yuki, H.; Okamoto, Y.; Kobayashi, Y. *J. Polym. Sci., Polym. Chem. Ed.* **1979**, *17*, 3867. (b) Galeazzi, R.; Geremia, S.; Mobbili, G.; Orena, in *M. Electron. Conf. Trends Org. Chem.*, Meeting Date 1995, Rzepa, H.; Leach, C.; Goodman, J. M., Eds. Royal Society of Chemistry: Cambridge, 1996; Paper 35.
(11) Kaiwar, V.; Reese, C. B.; Gray, E. J.; Neidle, S. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2281. Some modifications in the procedure allowed us to prepare a 50 g amount of cyclobutanone **3** in 70% yield from 3-(benzyloxy)prop-1-ene.
(12) Gagnon, R.; Grogan, G.; Groussain, E.; Pedragosa-Moreau, S.; Richardson, P. F.; Roberts, S. M.; Willetts, A. J.; Alphand, V.; Lebreton, J.; Furstoss, R. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2527.
(13) It is interesting to notice that the yield and ee were better than those previously described: Takabe, K.; Tamaka, M.; Sugimoto, M.; Yamada, T.; Yoda, H. *Tetrahedron: Asymmetry* **1992**, *3*, 1385.
(14) (a) Marino, J. P. *Pure Appl. Chem.* **1993**, *65*, 667. (b) Hart, D. J.; Hong, W. P.; Hsu, L. Y. *J. Org. Chem.* **1987**, *52*, 4665. (c) Davey, D. D. *J. Org. Chem.* **1987**, *52*, 4379. (d) Hogberg, T.; Ebner, M.; Ramsby, S.; Strom, P. *J. Org. Chem.* **1987**, *52*, 2033. (e) Dolle, R. E.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1985**, *107*, 1695.

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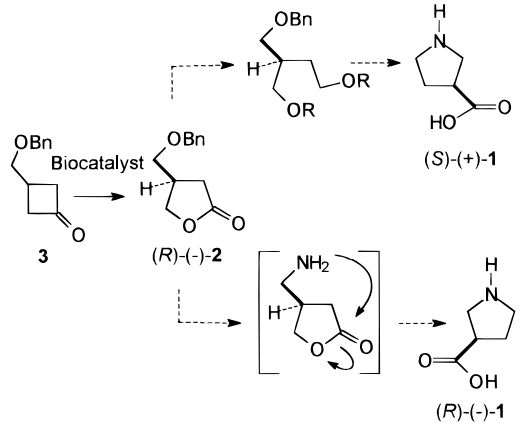
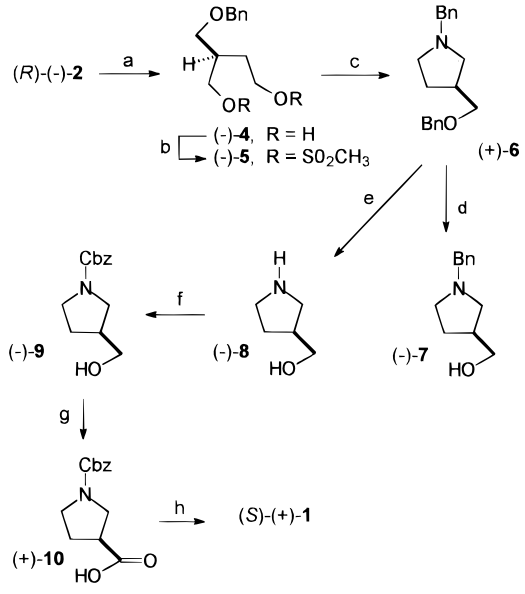
Scheme 1. Enantiodivergent Strategy for the Synthesis of *β*-Proline 1 Enantiomers



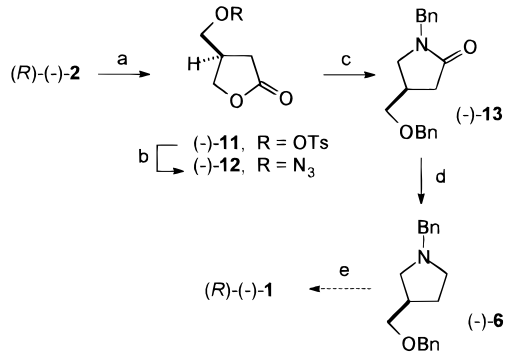
Scheme 2.[a] Synthesis of (*S*)-(+)-*β*-Proline 1



[a] Reaction conditions: (a) LAH, THF, 0 °C to reflux, 95%; (b) MsCl, DMAP, Et_3N, CH_2Cl_2, 0 °C, 95%; (c) $BnNH_2$, Et_3N, 1,4-dioxane, 70 °C, 85%; (d) H_2, Pd−C, MeOH, 95%; (e) H_2, Pd−C, EtOH, 95%; (f) BnOCOCl, K_2CO_3, THF−H_2O, 0 °C, 90%; (g) CrO_3, acetone, 90%; (h) H_2, Pd−C, MeOH, 95%.

N-benzylpyrrolidinol (*S*)-(−)-**7** as the sole product whereas, using ethanol instead of methanol, this reaction led directly to the free amino alcohol (*S*)-(−)-**8**. Surprisingly, however, all our efforts to oxidize alcohol (*S*)-(−)-**7** into the corresponding acid were unsuccessful, although several methods were tried.[2,3e,15] In order to overcome this difficulty, we decided to change the nature of the N-protecting group.[2] Thus, the free amino alcohol (*S*)-(−)-**8** was transformed into its *N*-carbamate (*S*)-(−)-**9** which was again submitted to oxidation. We were pleased to observe that this intermediate was indeed very smoothly and efficiently oxidized, using Jones procedure,[2] into the corresponding acid (*S*)-(+)-**10**.[16] Finally, hydrogenolysis of (*S*)-(+)-**10** afforded the desired (*S*)-(+)-*β*-proline **1**,[7] with an enantiomeric purity as high as that of the starting chiral lactone (*R*)-(−)-**2** (i.e., ee ≥ 97% chiral GC). Moreover, this last step was particularly interesting since it allowed to obtain directly, simply by filtration and stripping off the solvent, the amino acid target **1**. The overall yield from (*R*)-(−)-**2** was 55% for this seven-step synthesis.

Scheme 3.[a] Synthesis of (*R*)-(−)-*β*-Proline 1



[a] Reaction conditions: (a) (i) H_2, Pd−C, Et_2O; (ii) TsCl, Et_3N, CH_2Cl_2, +4 °C, 90% overall; (b) NaN_3, DMF, 70 °C, 90%; (c) (i) H_2, Pd−C, THF; (ii) NaH, BnBr, THF, 0 °C to reflux, 75% overall; (d) LAH, THF, 0 °C to reflux, 90%; (e) see Scheme 2.

Synthesis of (*R*)-(−)-**1** is depicted in Scheme 3. Cleavage of the benzyl group in (*R*)-(−)-**2**, followed by *in situ* tosylation, led to (*S*)-(−)-**11**[17] which was further transformed into the azide (*R*)-(−)-**12**. Reduction of (*R*)-(−)-**12** gave the primary amine (see Scheme 1), which was not isolated but was directly treated (at 0 °C) with sodium hydride and benzyl bromide, to afford the lactam (*R*)-(−)-**13**. Thus, through an internal rearrangement of the amine intermediate, we accomplished the desired stereochemical inversion, allowing for the synthesis of (*R*)-(−)-**1**, the other *β*-proline enantiomer.

Indeed, reduction of (*R*)-(−)-**13** by lithium aluminum hydride gave the desired dibenzyl pyrrolidine derivative (*R*)-(−)-**6** which, following the four-step reaction sequence depicted for (*S*)-(+)-**1**, led to the (*R*)-(−)-*β*-proline **1** antipode,[10a] with an enantiomeric purity as high as that of the starting chiral lactone (*R*)-(−)-**2**. The overall yield of this nine steps synthesis was about 40%.

In conclusion, we have described a new strategy allowing for the synthesis of both *β*-proline **1** enantiomers *via* a common chiral starting material, i.e. lactone (*R*)-(−)-**2**, obtained using a microbiological stereoselective Baeyer−Villiger oxidation. Moreover, the possibility to chemically differentiate the alcohol and amine functions of dibenzyl pyrrolidinol **6** makes each enantiomer of this compound an interesting chiral building block for the synthesis of other optically active pyrrolidine analogues. In this context, synthesis of both antipodes of homo-*β*-proline, as well as of other derivatives, are underway in our laboratory and will be presented elsewhere.

Experimental Section

General Methods. *C. echinulata* NRRL 3655 was obtained from the Culture Collection, Northern Regional Research Labo-

(15) (a) Mancuso, A. J.; Huang, S-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480. (b) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647. (c) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399. (d) Cella, J. A.; Kelley, J. A.; Kenehan, E. F. *J. Org. Chem.* **1975**, *40*, 1860. (e) Noureldin, N. A.; Lee, D. G. *Tetrahedron Lett.* **1981**, 4889. (f) Frigerio, M.; Santagostino, M.; Sputore, S.; Palmisano, G. *J. Org. Chem.* **1995**, *60*, 7272. (g) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.

(16) It should be mentioned that NMR experiments showed (*S*)-(−)-**9** and (*S*)-(+)-**10** do exist as 1:1 mixtures of the *cis* and *trans* conformers rotation (around the amide bond).

(17) Mori, K. *Tetrahedron* **1983**, *39*, 3107.

(18) The ee of (*R*)-(−)-**2** was determined by GC analysis using a Lipodex E (25 m × 0.25 mm, Macherey-Nagel) capillary column at 155 °C: (*R*)-(−)-**2** t_R = 82 min, (*S*)-(+)-**2**: t_R = 84 min.

ratory (USA). Microorganism cultures were carried out in a Setric 7 L fermentor. All reactions involving anhydrous conditions were conducted in flame-dried glassware under a positive atmosphere of oxygen-free nitrogen. All the dried solvents were distilled under nitrogen immediately prior use. THF, Et_2O, and CH_2Cl_2 were dried by distillation, respectively, from sodium benzophenone ketyl, on lithium aluminum hydride and on CaH. Separation by flash chromatography were performed using silica gel 60H and a pentane/ethyl acetate gradient. Melting points are uncorrected. ^{1}H NMR (250 MHz) and ^{13}C NMR (62.5 MHz) spectra were recorded in $CDCl_3$ (unless otherwise specified) with TMS as internal standard. Coupling constants (J) are in hertz. IR spectra were achieved in KBr film.

(*R*)-(−)-4-[(Benzyloxy)methyl]-dihydrofuran-2-one (2).[12,13] An adequate volume of suspension containing 2×10^7 spores (in 0.5% Tween 80 solution) of *C. echinulata* NRRL 3655 was used to inoculate a 5 L complex medium in a 7 L Setric fermentor (complex medium composition: 100 g of corn steep liquor (Roquette SA), 20 g of glucose, 5 g of KH_2PO_4, 10 g of K_2HPO_4, 10 g of $NaNO_3$, 2.5 g of KCl, 2.5 g of $MgSO_4$, 0.1 g of $FeSO_4$, 1 mL of Pluronic PE 8100 (BASF), 0.25 mL of Antifoam Silicon 426R (Prolabo). Cells were grown for 60 h at 27 °C (450 rpm, 30 L/h air) and then harvested by filtration and washed with water (5−7 g/L dry weight). They were suspended in 5 L of phosphate buffer (5 g of KH_2PO_4, 10 g of K_2HPO_4, pH 7). To this cell suspension was added an alcoholic solution of ketone **3** (5 g, 26 mmol, dissolved in 50 mL of EtOH) in the 7 L fermentor (27 °C, 450 rpm, 30 L/h air). The reaction was stopped after 24−30 h, once the ketone disappeared (reaction monitored by GC-BP 10 column). Biotransformation was quenched by addition of a HCl solution until pH 2. The medium was then continuously extracted with $CHCl_3$ for 48 h. After drying over $MgSO_4$, purification by flash chromatography afforded a mixture of 3-[(benzyloxy)methyl]cyclobutanols (*cis*/*trans:* 12/1, 0.8 g, 16%), identified by comparison with literature data,[11] and (*R*)-(−)-**2** (3.2 g, 60%), as a colorless oil: $[\alpha]^{19}_D = -36.8$ ($c = 1.4$, $CHCl_3$, ee ≥ 97%[18]) (lit.[13] $[\alpha]^{23}_D$ for (*S*)-(+)-**2** = +32.5 ($c = 0.9$, $CHCl_3$, ee = 95%)); ^{1}H NMR δ 2.45 (dd, $J = 17.6$, 6.3, 1H), 2.69 (dd, $J = 17.6$, 8.9, 1H), 2.85−3.00 (m, 1H), 3.52−3.60 (m, 2H), 4.26 (dd, $J = 9.2$, 5.5, 1H), 4.48 (dd, $J = 9.2$, 7.5, 1H), 4.60 (s, 2H), 7.33−7.48 (m, 5H); ^{13}C NMR δ 31.06, 35.28, 70.41, 70.73, 73.19, 127.60, 127.80, 128.44, 137.70, 176.92; IR (ν) 2862, 1774, 1365, 1172, 1101, 1023, 741, 700 cm^{-1}; MS EI (m/z) 206 (4), 177 (5), 160 (3), 120 (27), 105 (11), 91 (100), 65 (21), 39 (13), 27 (2). Anal. Calcd for $C_{12}H_{14}O_3$ (206.2436): C, 69.88; H, 6.84. Found: C, 69.63; H, 6.97.

(*S*)-(−)-2-[(Benzyloxy)methyl]butane-1,4-diol (4). To a suspension of lithium aluminum hydride (1.9 g, 50 mmol) in 10 mL of dry THF, at 0 °C, was added dropwise a solution of (*R*)-(−)-**2** (2.06 g, 10 mmol) in 20 mL of dry THF was added dropwise. The mixture was stirred 30 min at rt, refluxed for 1 h, cooled at 0 °C, diluted with Et_2O (50 mL), and quenched with water (5 mL). The solid was filtered off and washed again with water (20 mL). The organic phase was dried on $MgSO_4$ and concentrated to give, after flash chromatography, (*S*)-(−)-**4** (2.0 g, 95%) as an undistillable colorless oil: $[\alpha]^{25}_D = -8.4$ ($c = 2.1$, CH_2Cl_2); ^{1}H-NMR δ 1.63 (dd, $J = 11.9$, 6.5, 2H), 1.94−2.08 (m, 1H), 2.99 (s/br, 2H), 3.50 (m, 1H), 3.53 (m, 1H), 3.63−3.74 (m, 4H), 4.52 (s, 2H), 7.29−7.39 (m, 5H); ^{13}C-NMR δ 32.99, 39.85, 61.26, 65.31, 73.29, 73.98, 128.09, 128.31, 129.09, 138.56; IR (ν) 3342, 2867, 1454, 1364, 1266, 1206, 1062, 737, 699, 612 cm^{-1}; MS EI (m/z) 210 (0.5), 192 (2), 107 (25), 91 (100), 65 (23), 31 (22), 29 (10). Anal. Calcd for $C_{12}H_{18}O_3$ (210.2755): C, 68.53; H, 8.64. Found: C, 68.35; H, 8.86.

(*R*)-(−)-2-[(Benzyloxy)methyl]-1,4-bis(methansulfonyloxy)butane (5). To a solution of (*S*)-(−)-**4** (1.9 g, 9.0 mmol), (dimethylamino)pyridine (120 mg, 1 mmol), and Et_3N (2.7 g, 27 mmol) in dry CH_2Cl_2 (10 mL) was added mesyl chloride (2.6 g, 22.5 mmol) at 0 °C, and the mixture was stirred for 30 min and quenched with ice (2 g). The crude mixture was washed with water (10 mL) and 5% $NaHCO_3$ aqueous solution (10 mL). The organic layer was dried on $MgSO_4$, filtered off, and concentrated *in vacuo*, to give, after flash chromatography (*R*)-(−)-**5** (3.13 g, 95%) as an undistillable colorless oil: $[\alpha]^{25}_D = -4.9$ ($c = 3.1$, CH_2Cl_2); ^{1}H-NMR δ 1.90 (dd, $J = 12.9$, 6.4, 2H), 2.22−2.32 (m, 1H), 2.99 (s, 6H), 3.50 (d, $J = 1.9$, 1H), 3.52 (d, $J = 1.2$, 1H), 4.27−4.34 (m, 4H), 4.51 (s, 2H), 7.29−7.42 (m, 5H); ^{13}C-NMR δ 28.37, 36.19, 37.70, 37.94, 68.14, 69.33, 70.26, 73.93, 128.31, 128.50, 129.12, 138.43; IR (ν) 1358, 1265, 1176, 945, 738, 704, 528 cm^{-1}; MS EI (m/z) 366 (1), 270 (32), 179 (13), 145 (64), 107 (56), 91 (100), 79 (65), 68 (63), 55 (43), 41 (32), 29 (19). Anal. Calcd for $C_{14}H_{22}O_7S_2$ (366.4552): C, 45.88; H, 6.06. Found: C, 46.12; H, 6.31.

(*S*)-(+)-*N*-Benzyl-3-[(benzyloxy)methyl]pyrrolidine (6). Dimesylate (*R*)-(−)-**5** (3.0 g, 8.2 mmol), benzylamine (1.8 g, 16.5 mmol), and triethylamine (3 mL) in dioxane (30 mL) were refluxed for 12 h. The crude mixture was concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2 (50 mL), washed with 5% HCl solution (2 × 10 mL), concentrated, and purified by flash chromatography and bulb-to-bulb distillation (120 °C/0.1 mbar) to give (*S*)-(+)-**6** (2.0 g, 85%) as a colorless oil: $[\alpha]^{25}_D = +0.43$ ($c = 4.0$, CH_2Cl_2); ^{1}H-NMR δ 1.42−1.55 (m, 1H), 1.89−2.04 (m, 1H), 2.29 (dd, $J = 9.2$, 5.9, 1H), 2.44−2.62 (m, 2H), 2.72 (dd, $J = 9.0$, 7.8, 1H), 3.39 (d, $J = 7.2$, 2H), 3.59 (d, $J = 2.8$, 2H), 3.78−3.81 (m, 1H), 7.23−7.37 (m, 10H); ^{13}C-NMR δ 28.25, 38.07, 54.44, 58.22, 61.15, 73.63, 74.72, 127.48, 128.13, 128.22, 128.82, 129.01, 129.43, 139.17, 139.85; IR (ν) 3062, 3028, 2956, 2924, 2854, 2789, 1494, 1453, 1364, 1453, 1364, 1264, 1097, 1028, 737, 637 cm^{-1}; MS EI (m/z) 281 (3), 190 (24), 175 (65), 132 (10), 98 (17), 91 (100), 84 (48), 65 (19), 42 (19). Anal. Calcd for $C_{19}H_{23}NO$ (281.4013): C, 81.10; H, 8.24; N, 4.98. Found: C, 81.42; H, 8.33; N, 4.75.

(*S*)-(−)-*N*-Benzyl-3-(hydroxymethyl)pyrrolidine (7).[7] A suspension of (*S*)-(+)-**6** (0.5 g, 1.8 mmol) and 10% Pd/C (200 mg) was stirred 24 h in MeOH under H_2 (1 atm). The palladium carbon catalyst was filtered off, the solution concentrated, and the residue distilled (110 °C/0.1 mbar) to give (*S*)-(−)-**7** (0.32 g, 95%) as a colorless oil: $[\alpha]^{25}_D = -3.1$ ($c = 4.1$, EtOH) (lit.[7] $[\alpha]^{20}_D = -2.55$ ($c = 3.98$, EtOH)); ^{1}H-NMR δ 1.25−1.42 (m, 1H), 1.70−1.90 (m, 1H), 1.95 (s/br, 1H), 2.22−2.40 (m, 1H), 2.61 (dd, $J = 11.1$, 5.7, 1H), 2.71−2.90 (m, 2H), 2.90−2.99 (m, 1H), 3.20−3.40 (m, 2H), 4.44 (s, 2H), 7.25−7.35 (m, 5H); ^{13}C-NMR δ 30.07, 39.78, 47.55, 51.28, 73.62, 74.08, 128.09, 128.13, 128.96, 139.05; IR (ν) 3461, 3030, 2939, 2866, 1635, 1351, 1171, 1100, 940, 840, 736, 701, 529 cm^{-1}; MS CI (m/z) 192 ($M^+ + 1$, 100), 107 (35), 100 (35), 91 (84), 85 (96), 68 (35), 43 (96), 28 (80), 18 (50). Anal. Calcd for $C_{12}H_{17}NO$ (191.2754): C, 75.35; H, 8.96; N, 7.32. Found: C, 75.48; H, 9.14; N, 7.18.

(*S*)-(−)-3-(Hydroxymethyl)pyrrolidine (8).[7] A suspension of (*S*)-(+)-**6** (1.9 g, 6.7 mmol) and 10% Pd/C (200 mg), in EtOH was stirred 24 h under H_2 (1 atm). The palladium carbon catalyst was filtered off, the solution concentrated *in vacuo*, and the residue distilled (100 °C/0.1 mbar) to give (*S*)-(−)-**8** (0.64 g, 95%) as a colorless oil: $[\alpha]^{25}_D = -26$ ($c = 6.0$, EtOH) (lit.[7] $[\alpha]^{20}_D = -19.1$ ($c = 4.6$, EtOH)); ^{1}H-NMR δ 1.32−1.46 (m, 1H), 1.74−1.88 (m, 1H), 2.13−2.32 (m, 1H), 2.62−2.71 (m, 1H), 2.74−2.98 (m, 3H), 4;35 (s/br, 2H), 3.34−3.54 (m, 2H); ^{13}C-NMR δ 29.40, 41.93, 47.15, 50.51, 65.11; IR (ν) 3394, 1522, 1435, 762 cm^{-1}; MS EI (m/z) 101 (12), 82 (10), 68 (13), 55 (9), 43 (100), 28 (30), 15 (3). Anal. Calcd for $C_5H_{11}NO$ (101.1495): C, 59.36; H, 10.98; N, 13.85. Found: C, 59.52; H, 10.63; N, 13.67.

(*S*)-(−)-N-[(Benzyloxy)carbonyl]-3-(hydroxymethyl)pyrrolidine (9). Amino alcohol (*S*)-(−)-**8** (0.55 g, 5.4 mmol) and K_2CO_3 (3.7 g, 27 mmol) were dissolved in THF/H_2O (3/1, 100 mL) and treated with (benzyloxy)carbonyl chloride (1.9 g, 11 mmol) at 0 °C under stirring for 4 h. The reaction mixture was concentrated, and the residue was dissolved in CH_2Cl_2 (200 mL), washed with water (20 mL) and brine (10 mL), and concentrated *in vacuo*. After flash chromatography, (*S*)-(−)-**9** (1.14 g, 90%) was obtained as an undistillable colorless oil: $[\alpha]^{25}_D = -15$ ($c = 11.5$, $CHCl_3$); ^{1}H-NMR δ 1.44−1.64 (m, 1H), 1.70−1.92 (m, 1H), 2.15−2.38 (m, 1H), 3.05−3.12 (m, 1H), 3.15−3.32 (m, 1H), 3.32−3.50 (m, 4H), 3.79 (s/br, 1H), 4.99 (s, 2H), 7.08−7.35 (m, 5H); ^{13}C-NMR δ 27.75, 28.48, 40.84, 41.67, 45.75, 46.13, 49.01, 49.34, 64.19, 67.16, 128.15, 128.34, 128.86, 137.25, 155.49; IR (ν) 3422, 2949, 2879, 1686, 1426, 1360, 1128, 910, 733 cm^{-1}; MS EI (m/z) 235 (48), 190 (18), 160 (3), 144 (16), 128 (41), 114 (12), 100 (22), 91 (100), 65 (38), 55 (15), 41 (17), 28 (20). Anal. Calcd for $C_{13}H_{17}NO_3$ (235.2853): C, 66.35; H, 7.29; N, 5.95. Found: C, 66.13; H, 7.51; N, 5.72.

(*S*)-(+)-*N*-[(Benzyloxy)carbonyl]-3-carboxypyrrolidine (10). Alcohol (*S*)-(−)-**9** (1.04 g, 4.4 mmol) dissolved in acetone (50 mL) was treated dropwise with freshly prepared Jones reagent under vigorous stirring at 0 °C until a lasting orange color was obtained. 2-Propanol (0.5 mL) was added and the mixture filtered through Celite and washed with acetone/acetic

acid (10:1, 20 mL). The solvents were evaporated, and the residue was purified by flash chromatography to give (*S*)-(+)-**10** (1.0 g, 90%) as an undistillable colorless oil: $[\alpha]^{25}_D = +8.0$ (*c* = 5.0, $CHCl_3$); ^{1}H-NMR δ 2.15−2.36 (m, 2H), 3.00−3.27 (m, 1H), 3.40−3.85 (m, 4H), 5.25 (s, 2H), 7.28−7.55 (m, 5H), 10.9 (s/br, 1H); ^{13}C-NMR δ 28.54, 29.23, 42.65, 43.48, 45.70, 46.12, 48.30, 48.81, 67.61, 128.35, 128.50, 128.95, 137.03, 155.44, 177.32, 177.51; IR (ν) 3472, 2958, 1704, 1429, 1363, 1178, 1124, 737 cm^{-1}; MS EI (*m*/*z*) 249 (53), 204 (11), 158 (25), 142 (23), 115 (12), 114 (45), 107 (15), 91 (100), 65 (53), 41 (23), 27 (4). Anal. Calcd for $C_{13}H_{15}NO_4$ (249.2688): C, 62.63; H, 6.08; N, 5.62. Found: C, 62.81; H, 6.29; N, 5.86.

(*S*)-(+)-3-Carboxypyrrolidine (1).[7] A suspension of (*S*)-(+)-**6** (0.9 g, 0.36 mmol) and 10% Pd/C (100 mg) in MeOH was stirred 24 h under H_2 (1 atm). The palladium carbon catalyst was filtered off, the solution concentrated *in vacuo*, and the residue crystallized to give (*S*)-(+)-**1** (0.4 g, 95%) as a white solid: mp 193−195 °C (lit.[7] mp 188−190 °C); $[\alpha]^{25}_D = +19.1$ (*c* = 2.0, H_2O) (lit.[7] $[\alpha]^{20}_D = +18.5$ (*c* = 3.14, H_2O)); ^{1}H-NMR (CD_3OD) δ 2.06−2.36 (m, 2H), 3.20−3.36 (m, 3H), 3.40−3.54 (m, 2H), 5.04 (s/br, 2H); ^{13}C-NMR (CD_3OD) δ 29.47, 43.50, 46.71, 48.46, 176.50; IR (ν) 3382, 2955, 27.58, 1728, 1574, 1403, 1225, 1041 cm^{-1}.

(*S*)-(−)-4-[(*p*-Toluensulfonyloxy)methyl]-dihydrofuran-2-one (11).[17] A suspension of lactone (*R*)-(−)-**2** (2.5 g, 12 mmol) and 10% Pd/C (200 mg) in Et_2O (30 mL) was stirred 24 h under H_2 (1 atm). The palladium carbon catalyst was filtered off, the solution concentrated *in vacuo*, and the residue dissolved in CH_2Cl_2 (12 mL) and triethylamine (2.5 mL). Solid TsCl (3.5 g, 18 mmol) was added over 5 min at 0 °C, and the reaction mixture was left overnight at +4 °C and quenched, at the end, with ice (1 g). The organic layer was separated, washed with 5% HCl aqueous solution and brine and concentrated to give, after flash chromatography, (*S*)-(−)-**11** (2.9 g, 90% overall) as a white solid: mp 55−56 °C; $[\alpha]^{25}_D = -30$ (*c* = 4.1, $CHCl_3$); ^{1}H-NMR δ 2.28 (dd, *J* = 17.8, 6.4, 1H), 2.46 (s, 3H), 2.63 (dd, *J* = 17.8, 9.1, 1H), 2.87−3.03 (m, 1H), 4.03−4.09 (m, 3H), 4.38 (dd, *J* = 9.6, 7.6, 1H), 7.39 and 7.78 (d's, *J* = 8, 2H each); ^{13}C-NMR δ 22.21, 30.99, 35.13, 69.98, 70.07, 128.41, 130.72, 132.70, 146.10, 176.11; IR (ν) 3060, 2984, 2918, 1779, 1598, 1363, 1267, 1177, 1097, 1028, 974, 830, 738, 667, 565 cm^{-1}; MS EI (*m*/*z*) 270 (6), 206 (51), 202 (22), 173 (9), 155 (25), 143 (16), 127 (15), 117 (19), 107 (32), 91 (100), 87 (26), 65 (32), 47 (43), 35 (35). Anal. Calcd for $C_{12}H_{14}O_5S$ (270.3064): C, 53.31; H, 5.23. Found: C, 53.54; H, 5.37.

(*R*)-(−)-4-(Azidomethyl)-dihydrofuran-2-one (12). Tosylate (*S*)-(−)-**11** (2.8 g, 10.3 mmol) and sodium azide (1.2 g, 22 mmol) in DMF (10 mL) were stirred at 70 °C overnight. The mixture was poured into a saturated NaCl aqueous solution (15 mL) and extracted with Et_2O (50 mL). After evaporation of the solvents and purification by flash chromatography, (*R*)-(−)-**12** (1.3 g, 90%) was obtained as an undistillable colorless oil: $[\alpha]^{25}_D = -44$ (*c* = 3.2, $CHCl_3$); ^{1}H-NMR δ 2.36 (dd, *J* = 17.5, 6.2, 1H), 2.68 (dd, *J* = 17.5, 8.7, 1H), 2.74−2.90 (m, 1H), 3.42−3.55 (m, 2H), 4.13 (dd, *J* = 9.4, 5.5, 1H), 4.43 (dd, *J* = 9.4, 7.3, 1H); ^{13}C-NMR δ 32.49, 35.82, 53.55, 71.11, 176.48; IR (ν) 30.56, 2105, 1779, 1674, 1266, 1177, 1027, 738, 704 cm^{-1}; MS EI (*m*/*z*) 142 ($M^+ + 1$, 86), 112 (14), 96 (39), 85 (43), 68 (100), 55 (84), 39 (94), 29 (92). Anal. Calcd for $C_5H_7N_3O_2$ (141.1304): C, 42.54; H, 5.01; N, 29.77. Found: C, 42.30; H, 5.28; N, 29.91.

(*R*)-(−)-*N*-Benzyl-4-[(Benzyloxy)methyl]pyrrolidin-2-one (13). A suspension of (*R*)-(−)-**12** (1.2 g, 8.5 mmol) and 10% Pd/C (200 mg) in THF (30 mL) was stirred 2 days under hydrogen (1 atm). The palladium carbon catalyst was rapidly filtered off and the reaction mixture treated at 0 °C with 60% NaH oil suspension (4.1 g, 85 mmol). After 10 min, benzyl bromide (4.3 g, 25.5 mmol) was added at once, and the mixture was stirred for 1 h more at 0 °C, refluxed for 3 h, and quenched at 0 °C with wet Et_2O (50 mL) and water (2 mL). The organic layer was washed with 5% HCl aqueous solution (10 mL), and brine and concentrated to give, after flash chromatography, (*R*)-(−)-**13** (1.88 g, 75% overall) as an undistillable colorless oil: $[\alpha]^{25}_D = -4.2$ (*c* = 2.8, CH_2Cl_2); ^{1}H-NMR δ 2.10−2.35 (m, 1H), 2.45−2.65 (m, 2H), 2.95−3.10 (m, 1H), 3.20−3.50 (m, 3H), 4.36 and 4.39 (s's, 2H each), 7.05−7.50 (m, 10H); ^{13}C-NMR δ 31.72, 34.79, 46.99, 50.02, 72.65, 73.70, 128.14, 128.26, 128.62, 128.70, 128.95, 129.17, 136.94, 138.44, 174.29; IR (ν) 2923, 2854, 1684, 1495, 1453, 1259, 1101, 738 cm^{-1}; MS EI (*m*/*z*) 295 (21), 204 (20), 189 (24), 146 (7), 118 (6), 91 (100), 65 (20), 41 (10), 28 (12). Anal. Calcd for $C_{19}H_{21}NO_2$ (295.3847): C, 77.23; H, 7.17; N, 4.74. Found: C, 77.46; H, 7.28; N, 4.52.

(*R*)-(−)-*N*-Benzyl-3-[(benzyloxy)methyl]pyrrolidine (6). To a suspension of lithium aluminum hydride (1.0 g, 26 mmol) in dry THF (10 mL), at 0 °C, a solution of (*R*)-(−)-**13** (1.8 g, 6.1 mmol) in dry THF (20 mL) was added dropwise. The mixture was stirred 30 min at rt and refluxed for 2 h. It was then cooled at 0 °C, diluted with wet Et_2O (50 mL), and quenched with water (5 mL), the solid filtered off, and the filtrate washed with water, dried on $MgSO_4$, and concentrated to give, after flash chromatography, (*R*)-(−)-**6** (1.56 g, 90%) as a colorless oil: $[\alpha]^{25}_D = -0.49$ (*c* = 4.1, CH_2Cl_2). Anal. Calcd for $C_{19}H_{23}NO$ (281.4013): C, 81.10; H, 8.24; N, 4.98. Found: C, 81.24; H, 8.46; N, 5.09.

(*R*)-(+)-3-(Hydroxymethyl)pyrrolidine (8). Starting from (*R*)-(−)-**6** (1.45 g, 5.1 mmol) and following the same procedure as for (*S*)-(−)-**8**, (*R*)-(+)-**8** (0.49 g, 95%) was obtained as a colorless oil: $[\alpha]^{25}_D = +24$ (*c* = 5.0, EtOH). Anal. Calcd for $C_5H_{11}NO$ (101.1495): C, 59.36; H, 10.98; N, 13.85. Found: C, 59.56; H, 11.13; N, 14.07.

(*R*)-(+)-*N*-[(Benzyloxy)carbonyl]-3-(hydroxymethyl)pyrrolidine (9). Starting from (*R*)-(+)-**8** (0.4 g, 4 mmol) and following the same procedure as for (*S*)-(−)-**9**, (*R*)-(+)-**9** (0.85 g, 90%) was obtained as an undistillable colorless oil: $[\alpha]^{25}_D = +16$ (*c* = 9.0, $CHCl_3$). Anal. Calcd for $C_{13}H_{17}NO_3$ (235.2853): C, 66.35; H, 7.29; N, 5.95. Found: C, 66.52; H, 7.55; N, 5.69.

(*R*)-(−)-*N*-[(Benzyloxy)carbonyl]-3-carboxypyrrolidine (10). Starting from (*R*)-(+)-**9** (0.8 g, 3.4 mmol) and following the same procedure as for (*S*)-(+)-**10**, (*R*)-(−)-**10** (0.76 g, 90%) was obtained as an undistillable colorless oil: $[\alpha]^{25}_D = -9.5$ (*c* = 4.3, $CHCl_3$). Anal. Calcd for $C_{13}H_{15}NO_4$ (249.2688): C, 62.63; H, 6.08; N, 5.62. Found: C, 62.85; H, 6.17; N, 5.45.

(*R*)-(−)-3-Carboxypyrrolidine (1).[10a] Starting from (*R*)-(−)-**10** (0.5 g, 2 mmol) and following the same procedure as for (*S*)-(+)-**1**, (*R*)-(−)-**1** (0.22 g, 95%) was obtained as a white solid: mp 185−187 °C (lit.[10a] mp 186−188.5 °C); $[\alpha]^{25}_D = -18.9$ (*c* = 2.0, H_2O) (lit.[10a] $[\alpha]^{20}_D = -18.7$ (*c* = 2.3, H_2O)).

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