Chemoenzymatic Synthesis of Isogalactofagomine

Xifu Liang, Anders Lohse, and Mikael Bols*

Department of Chemistry, University of Aarhus, DK-8000 Aarhus, Denmark

mb@kemi.aau.dk

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A new chemoenzymatic synthesis of optically pure isogalactofagomine **2** starting from achiral starting materials is presented. Dimethyl 4-hydroxypyridine-3,5-dicarboxylate (**7**) was synthesized and converted to the corresponding saturated piperidine **8**. Then the key step of the synthesis was carried out: Lipase M catalyzed hydrolysis of the prochiral diester **8** to cause formation of an asymmetric monoacid with at least 98% enantiomeric excess. Reduction of the acid, saponification of the remaining ester, and radical iododecarboxylation gave an iodide that after substitution with silver trifluoroacetate and hydrolysis gave **2**.

Introduction

Enzymes are useful catalysts for synthesis of enantiomerically pure compounds that can be used to create new chiral centers, resolve racemates or convert prochiral compounds to asymmetric molecules.[1] A particularly popular group of enzymes in organic synthesis is the esterases, proteases and lipases because they can be used on a wide range of substrates; furthermore, the latter subgroup can be used in organic solvents. These enzymes are typically used to resolve a racemic ester or alcohol by selective hydrolysis or esterification of one enantiomer or to desymmetrize a meso diester or diol by monohydrolysis or monoesterication. The latter procedure is clearly the more efficient, because all of the substrate can be converted to desired compound.

We here demonstrate the use of enzymes in the synthesis of the potent galactosidase inhibitor **2**. Compound **2** is the galactose analogue of the glucosidase inhibitor isofagomine (**1**)[2] and has been shown to be a very strong and selective β-galactosidase inhibitor.[3] This compound has previously been synthesized from carbohydrates[3] or in racemic form.[4] The former synthesis of optically pure **2** was rather lengthy (11 steps) and started from relatively expensive D-lyxose. Our new synthesis is shorter and more flexible and utilizes achiral starting materials.

Results and Discussion

The idea of this synthesis was to use enantioselective enzymatic hydrolysis of a prochiral diester as the key step. Such monohydrolysis reactions have frequently been found highly stereoselective with pig liver esterase (PLE) as catalyst,[5] and lipases have also been used.[6] Our attention was attracted to the substrate **3a** (Figure 1), which is hydrolyzed to the 3-monoacid with more than 95% ee by PLE.[5d] Some controversy has existed over the absolute configuration of this substance. Zemlicka et al., who first reported the reaction, assigned the acid to 3*S*,5*R* configuration based on X-ray crystallography.[5d] Subsequently this has been challenged by Roberts et al., however, who through conversion to a compound with known configuration convincingly argued that the product was the 3*R*,5*S* acid.[5e,f] This assignment is supported by the reports that the substrates **3b** and **4** are hydrolyzed by PLE with similar stereochemical preference (Figure 1). Thus the 2-deoxyderivative **3b** of the 1,3-dicarboxylic ester **3a** was hydrolyzed preferentially at the 3-ester with an enantiomeric excess of 60%,[5g] while the 2,4,5-trideoxy-4,5-nor-isopropylidine analogue **4** also was

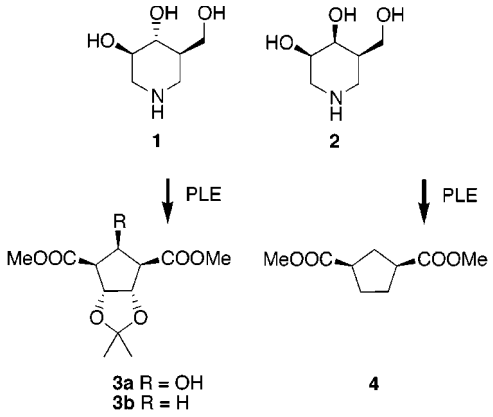

Figure 1. Isofagomine (**1**), the target **2**, and a prochiral PLE substrate **3a**, **3b**, and **4**. The arrow shows where PLE hydrolysis occur.

* Tel: +4589423963. Fax: +4586196199.

(1) (a) Faber, K. *Biotransformations in Organic Chemistry*; Springer-Verlag: Berlin, 1997. (b) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon Press: Oxford, 1994.

(2) Bols, M. *Acc. Chem. Res.* **1998**, *31*, 1−8.

(3) Ichikawa, Y.; Igarashi, Y.; Ichikawa M.; Suhura Y. *J. Am. Chem. Soc.* **1998**, *120*, 5854.

(4) Hansen, S. U.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2000**, 911−5.

(5) (a) Sabbioni, G.; Shea, M. L. Jones, J. B. *J. Chem. Soc., Chem. Commun.* **1984**, 236−8. (b) Schneider, M.; Engel, N. Honicke, P.; Heinemann, G.; Gorisch, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 67−8. (c) Bloch, R. Guibé-Jampel, E.; Girard, C. *Tetrahedron Lett.* **1985**, *26*, 4087−90. (d) Zemlicka, J.; Craine, L. E.; Heeg, M. J.; Oliver, J. P. *J. Org. Chem.* **1988**, *53*, 3 937−42. (e) Hutchinson, E. J.; Roberts, S. M.; Thorpe, A. J. *J. Chem. Soc., Perkin Trans 1* **1992**, 2245−6. (f) Hill, J. M.; Hutchinson, E. J.; Le Grand, D. M.; Roberts, S. M.; Thorpe, A. J.; Turner, N. J. *J. Chem. Soc., Perkin Trans 1* **1994**, 1483−7. (g) Hultin, P. G.; Mueseler, F.-J.; Jones, J. B. *J. Org. Chem.* **1991**, *56*, 5375−80. (h) Jones, J. B.; Hinks, R. S.; Hultin, P. G. *Can. J. Chem.* **1985**, *63*, 452−6.

(6) Guibé-Jampel, E.; Rousseau, G.; Salaun, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1080−1.

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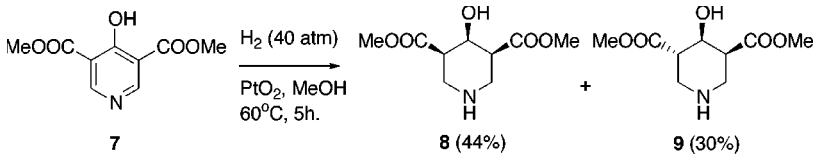
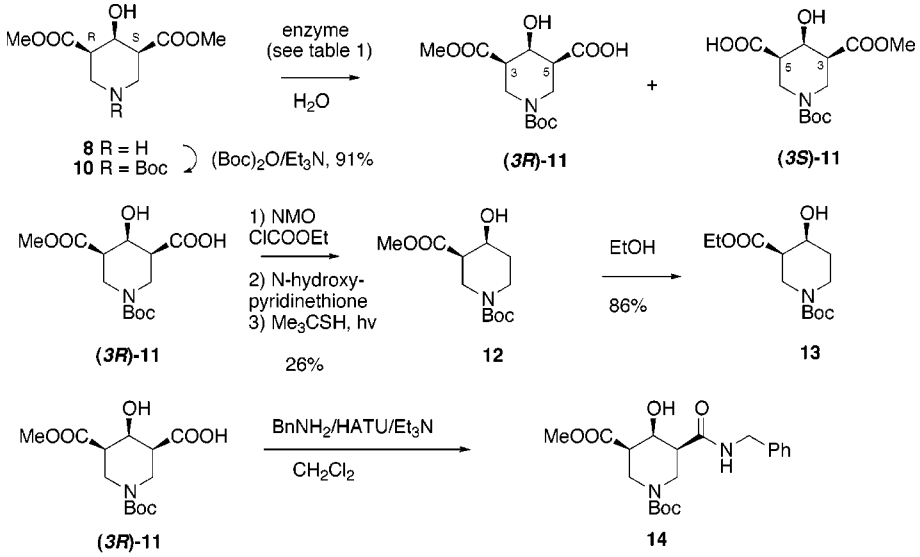
Scheme 1. Synthesis of Piperidinediester 8



Scheme 2. Enzyme-Catalyzed Hydrolysis of 10 and Preparation of Derivatives Used for Determination of Absolute Configuration and Enantiomeric Purity



hydrolyzed at the 3-ester with an enantiomeric excess of 20% (Figure 1).[5h]

Provided a similar transformation could be applied to the quite similar diester **8** (Scheme 1), then a reduction of the acid, hydrolysis, and oxidative decarboxylation of the ester would lead to **2**.

The diester **8** was synthesized from the known pyridine **7** (Scheme 1).[7] This was synthesized according to the published procedure. Hence, base-catalyzed condensation between *s*-triazine (**5**) and diethyl 3-ketoglutarate **6** gave crystalline **7**. The yield in this reaction was reported to be 56%,[7] but a lowering of pH during workup was found to increase the yield to 92%.

Saturation of the pyridine was expected to lead to the key diester **8**, and this was carried out by catalytic hydrogenation. The reaction did proceed at high pressure and temperature with a number of different catalysts (Rh, Pt) but was not as stereoselective as one might have hoped. Besides the all-*syn* product **8** other products were formed depending on the catalyst. The best result was obtained using PtO_2 and hydrogen at 40 atm at 60 °C in acetic acid.[8] This gave only two products, **8** and **9**, which could be separated and isolated in 44% and 30% yield, respectively. The all-*syn* product **8** was converted to the *N*-Boc derivative **10** by treatment with *tert*-butoxycarbonyl anhydride and triethylamine (Scheme 2).

Enzyme-catalyzed hydrolysis of **10** was now investigated. The PLE-catalyzed hydrolysis was expected to lead to hydrolysis of the *R* ester to give **(3*S*)-11** according to the results of Zemlicka et al.,[5d] while the work of Roberts et al. suggested that **(3*R*)-11** would be formed.[5e,f] PLE-catalyzed hydrolysis of **10** proceeded smoothly, and the product was found to be **(3*R*)-11**, which was obtained in the comparatively low optical purity of 79% enantiomeric excess (ee, Table 1). Configuration and optical purity was determined in the following way. The configuration of **(3*R*)-11** was found by converting it to the known compound **13**, which has been made by Bakers yeast reduction of the corresponding ketoester (Scheme 2).[9,10] Reacting the acid **(3*R*)-11** (79% ee) with ethyl chloroformate and *N*-methyl morpholine gave the acid chloride, which was reacted with N-hydroxypyridine-2-thione to the acyloxypyridine and finaly reductively decarboxylated with *tert*-butylmercaptan and light to give **12** in 26% yield over three steps. Transesterification using Na_2CO_3 in EtOH gave **13** in 86% yield.

Table 1. Enzyme-Catalyzed Hydrolysis of meso Compound 10

enzyme	time (days)	product (major)	ee[a] (%)	E
pig liver esterase (PLE)	1	**(3*R*)-11**	79	8.5
lipase SP 524 (*Mucor miehei*)	4	**(3*R*)-11**	83	10.8
lipase SP 523 (*Humicola thermo*)	2	**(3*R*)-11**	91	21.2
lipase F (*Rhizopus sp.*)	2	**(3*R*)-11**	93	27.6
lipase lipozym (*Mucor miehei*)	3	**(3*R*)-11**	93	27.6
lipase AP (*Aspergillus niger*)	1	**(3*R*)-11**	89	17.2
lipase AY (*Candida rugosa*)	8	**(3*R*)-11**	94	32.3
lipase N (*Rhizopus niveus*)	7	**(3*R*)-11**	94	32.3
lipase D (*Rhizopus delemar*)	2	**(3*R*)-11**	98	99
lipase M (*Murcor javanicus*)	2	**(3*R*)-11**	99	199
subtilisin Carlsberg	9	**(3*S*)-11**	77	7.7

[a] The ee values of the monoacid were determined by HPLC (column, OD-column; eluent, *n*-hexane/2-propanol 5:1; flow rate, 0.5 mL/min; UV, 260 nm) after the monoacid was converted to benzylamide derivatives.

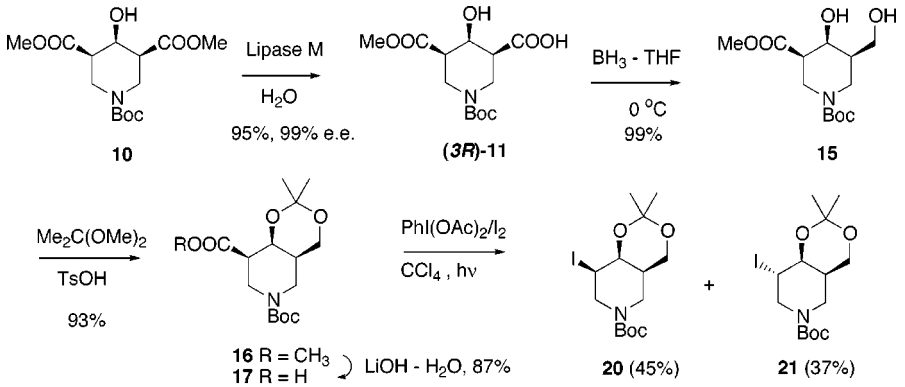
(7) Huffman, K. R.; Schaefer, F. C.; Peters, G. C. *J. Org. Chem.* **1962**, *27*, 551−8.

(8) Stetter, H.; Hennig, H. *Chem. Ber.* **1955**, *88*, 789−95.

(9) (a) Knight, D. W.; Lewis, N.; Share, A. C.; Haigh, D. *Tetrahedron: Asymmetry* **1993**, *4*, 625−28. (b) Knight, D. W.; Lewis, N.; Share, A. C.; Haigh, D. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3673−83.

(10) Willert, M.; Bols, M. *Acta Chem. Scand.* **1998**, *52*, 461−8.

Scheme 3. Synthesis of Iodides 20 and 21



The specific rotation of **13** (79% ee) was found to be +31.4° (*c* 0.66, CH_2Cl_2). For comparison we have previously found that **13** (41% ee) had a specific rotation of +23° (*c* 3.8, CH_2Cl_2).[10] Knight et al. has also found that **13** had a positive rotation.[9] Therefore the absolute configuration of **13** has been proved to be (3*R*,*4S*).

The ee of the product was determined by reaction with benzylamine, *N*-[(dimethylamino)-1*H*-1,2,3-triazole[4,5-b]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate (HATU) and triethylamine in CH_2Cl_2 to give the benzylamide **14** (Scheme 2), which was analyzed by chiral HPLC. With a chiralcel OD (acetylated cyclodextrin) column, fine separation of the enantiomers of **14** was observed (see Supporting Information).

Thus the stereoselectivity of PLE toward substrate **10** is similar to that of **3b** and **4**,[5g5h] and supports Roberts' structural assignment for the product of hydrolysis of **3a**.[5e,5f]

The configurational selectivity of the enzyme was not crucial for the synthesis since the desired target could be obtained by functional group manipulation. However, the enantioselectivity of the reaction had to be improved. We therefore screened a series of lipases and a protease for catalysis of the reaction **10** to **11** (Table 1). All of the lipases gave the same configurational selectivity as PLE but with higher enantiomeric excess. The direction of the selectivity may be explained by the socalled "Kazlauskas" rule, which predicts preferential hydrolysis of an ester with *S*- configuration at the α-carbon by lipases.[1a,11] The best result was obtained with lipase M from *Rhizopus delemar*. With that enzyme the ee was more than 99%, which should correspond to an enantiomer selectivity of more than 100 to 1.

The protease subtilisin Carlsberg gave the opposite configuration with about the same enantioselectivity as PLE. This result may be rationalized by comparison of the substrate **10** with an amino acid ester. Under the assumption that the carbon with the polar hydroxygroup prefers to bind to a polar site in the protease intended for binding the amino group of an amino acid, then hydrolysis of the *R* ester would correspond to hydrolysis of an L-amino acid ester, while hydrolysis of the *S* ester would correspond to hydrolysis of an D-amino acid ester (Figure 2). Clearly hydrolysis of the *R* ester should be preferred because proteases have great preference for L-amino acid derivatives.[1]

The synthesis was now continued by lipase M catalyzed hydrolysis of **10** (Scheme 3), which gave **(3*R*)-11** in 78% yield with an optical purity of more than 99% ee This compound was then subjected to selective reduction of the acid with borane−THF complex[12] at 0 °C to give the diol **15**. The diol moiety was protected as an isopropylidene derivative by reaction of **15** with dimethoxypropane and *p*-toluenesulfonic acid (TsOH). This gave **16** in a 92% yield from **(3*R*)-11**. The ester of **16** was hydrolyzed with LiOH in aqueous THF, which gave the acid **17** in 87% yield.

A number of different attempts were carried out to convert the carboxylic group of **16** or **17** into a hydroxyl function. The idea to convert **16** or **17** into a methyl ketone, which could be subjected to Baeyer−Villiger oxidation with *m*-chloroperbenzoic acid (MCPBA), failed because methyllithium addition to the carboxylate could not be limited to monoaddition and the tertiary alcohol was invariably formed. Baeyer−Villiger oxidation has

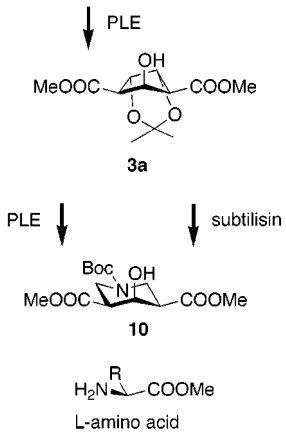


Figure 2. Comparison of the enzyme-catalyzed hydrolysis of various prochiral diesters. The arrows show where hydrolysis by the indicated enzyme occurs. The structure of **10** is also compared with an L-amino acid to explain the stereochemical preference of subtilisin-catalyzed hydrolysis.

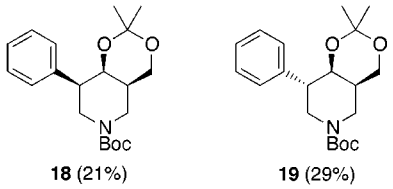
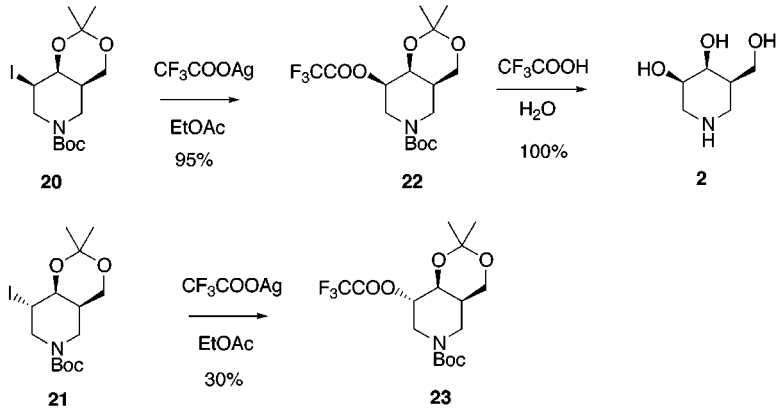


Figure 3. The products of $Pd(OAc)_4$-promoted decarboxylation of the carboxylic acid **17**.

(11) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656−65.

(12) Yoon, N. M.; Pak, C. S.; Brown, H. C.; Krishnamurthy, S.; Stocky, T. P. *J. Org. Chem.* **1973**, *38*, 2786−92.

Scheme 4. Synthesis of 2



also been reported to occur on aldehydes;[13] so **16** was reduced to the corresponding aldehyde with DIBAL-H, but the aldehyde was reoxidized to **17** when treated with (MCPBA). Neither did oxidation of **17** with $Pd(OAc)_4$ and KOAc in benzene[14] lead to any *O*-acetate, but the two phenyl derivatives **18** and **19** were isolated in low yield (Figure 3). These two products are formed by presumably formed by Friedel−Craft alkylation of the solvent by a piperidine carbocation. The lead oxidation is believed to give a radical that is oxidized to a carbocation, which in this case apparently prefers to add to benzene. Such byproducts have been observed previously.[15]

As radical decarboxylation had been successful in the conversion of **11** to **12** a Hunsdiecker-type decarboxylation was attempted. Acid **17** was treated with Suarez reagent[16] and light, which gave the two epimeric iodides **20** and **21** in almost equimolar amounts. They were separated by chromatography to give the *ribo* isomer **20** in 45% yield and the *arabino* isomer **21** in 37% yield, respectively. The configuration of the two iodides were determined by ^{1}H NMR spectroscopy. Compound **20** had a large coupling (J = 11.4 Hz) between H-3 and H-2ax, which **21** did not have. This shows that H-3 is axial in **20** and not in **21**, which is only consistent with **20** being the *ribo* isomer and **21** the *arabino* isomer.

Nucleophilic substitution of the two iodides with silver trifluoroacetate in EtOAc gave retention of configuration (Scheme 4). Thus reaction of **20** gave the *ribo* configured trifluoroacetate **22** with in 95% yield. This is very clearly seen from the ^{1}H NMR spectrum; it was very similar to that of **20** except that the double-double-doublet signal of H-3 (containing a large $J_{2ax,3}$) had moved from δ 4.1 to δ 4.7 (see Supporting Information). Compound **21** gave two products; one was the *arabino* trifluoroacetate **23**, and the other was unidentified (but not **22**). The retention stereochemistry of this reaction may seem surprising but is precedented by a number of examples.[17] It has been suggested that the reaction takes place by a S_Ni type mechanism with iodine abstraction and substitution occurs in a six-membered transition state occurring from the same side.[17b] However inversion of configuration can also occur,[18] so stereochemical assignment should be carried out with caution in this reaction.

Hydrolysis of **22** with aqueous trifluoroacetic acid gave a quantitative yield of isogalactofagomine **2**. The product had a ^{1}H NMR spectrum identical to previously published values.[3] The specific rotation of the product, $[\alpha]_D$ +2.5°, could not be compared as it has not been reported. Therefore as an extra characterization of the product the inhibition constant, K_i, was measured toward β-galactosidase from *Aspergillus orizae* and compared to the previously reported value of 4 nM.[3] A value of 3.2 nM was found.

In summary, we have reported a chemoenzymatic synthesis of the important inhibitor **2**. The synthesis starts from achiral starting materials, is 10 steps, and has an overall yield of 10%. For comparison, the previous asymmetric synthesis starts from D-lyxose, is 11 steps and has an overall yield of 5%.[3] Beside this improvement an achiral synthesis, this method has the advantage of greater flexibility in regard to synthesis of analogues.

Experimental Section

The general procedures were as previously described.[4]

4-Hydroxypyridine-3,5-dicarboxylic Acid Dimethylester (7). To a solution of dimethyl 1,3-acetonedicarboxylate **6** (15.0 g, 86.1 mmol) and NaOMe (4.8 g, 88.9 mmol) in MeOH (150 mL) was added *s*-triazine **5** (6.75 g, 83.3 mmol) at room temperature. After stirring for 10 min at the same temperature, the mixture was heated to reflux for 0.5 h, cooled to room temperature,and then neutralized with concentrated aqueous HCl. After the mixture stood for 2 h, the crystals were filtered off and washed with H_2O, MeOH, and Et_2O to provide **7** (16.2 g, 92%) as a colorless solid. Mp: 249−250 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.21 (s, 2H), 3.75 (s, 6H).

(3,4-*cis*-4,5-*cis*)-4-Hydroxypiperidine-3,5-dicarboxylic Acid Dimethylester Hydroacetate (8) and (±)-(3,4-*cis*-4,5-*trans*)-4-Hydroxypiperidine-3,5-dicarboxylic Acid Dimethyl Ester Hydroacetate (9). A mixture of **7** (1.00 g, 4.74 mmol) and PtO_2 (200 mg, 0.88 mmol) in AcOH (40 mL) was hydrogenated under 40 atm of H_2 for 5 h at 60 °C. Filtration through Celite and concentration provided a crude mixture of **8** and **9**, from which **8** was crystallized with ethyl acetate (0.58 g, 44%). The mother liquor was concentrated in vacuo and purified by chromatography (ethyl acetate/MeOH 5:1, R_f 0.16 for **9**) to provide **9** (0.40 g, 30%) as a colorless oil. Data for **8**. Mp: 162 °C. ^{1}H NMR (200 MHz, CD_3OD): δ 4.80 (t, 1H, J 2.4), 3.75 (s, 6H), 3.35−3.20 (m, 4H), 2.72 (ddd, 2H,

(13) (a) Wender, P. A.; Howbert, J. J. *Tetrahedron Lett.* **1983**, *24*, 5325−8. (b) Forster, P. G.; Ghisalbert, E. L. Jefferies, P. R. *Tetrahedron* **1987**, *43*, 2999−3007.

(14) Sheldon, R. A.; Kochi, J. K. *Org. React.* **1972**, *19*, 279−421.

(15) Aratani, T.; Nakanisi, Y.; Nozaki, H. *Tetrahedron Lett.* **1969**, 1809−10.

(16) Conception, J. I.; Francisco, C. G.; Freire, R.; Hernandez, R.; Salazar, J. A.; Suarez, E. *J. Org. Chem.* **1986**, *51*, 402−4.

(17) (a) Baker, R. K.; Rupprecht, K. M.; Armistead, D. M.; Boger, J.; Frankshun, R. A.; Hodges, P. J.; Hoogsteen, K.; Pisano, J. M.; Witzel, B. E. *Tetrahedron Lett.* **1998**, *39*, 229−32. (b) Bloodsworth, A. J.; Bowyer, K. J.; Mitchell, J. C. *J. Org. Chem.* **1987**, *52*, 1124−8.

(18) Clausen, R. P., Bols, M. *J. Org. Chem.* **2000**, *65*, 2797−801.

J 11.0, *J* 11.0, *J* 5.8, *J* 5.8), 2.00 (s, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 171.4, 64.9, 50.3, 46.0, 39.5, 22.3. HRMS (ES): calcd for $C_9H_{15}NO_5 + H^+$ 218.1028, found 218.1026. Data for **9**. ^{1}H NMR (200 MHz, CD_3OD): δ 4.80 (dd, 1H, *J* 3.3, *J* 5.2 Hz), 3.82/3.79 (s, 6H), 3.40−3.10 (m, 4H), 3.06−2.95 (m, 2H), 2.00 (s, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 172.6, 172.1, 65.7, 51.9, 51.5, 45.8, 43.3, 41.8, 41.4, 22.3. HRMS (ES): calcd for $C_9H_{15}NO_5 + H^+$ 218.1028, found 218.1025.

(3,4-*cis*-4,5-*cis*)-*N*-*tert*-Butyloxycarbonyl-4-hydroxypiperidine-3,5-dicarboxylic Acid Dimethyl Ester (10). To a solution of **8** (1.28 g, 4.62 mmol) and Et_3N (3 mL) in CH_2Cl_2 (30 mL) was added $(Boc)_2O$ (1.50 g, 6.87 mmol) at room temperature. After stirring for 1 h, the reaction solution was concentrated in vacuo. The residue was purified by chromatography (ethyl acetate/pentane 1:3, R_f 0.37) to provide **10** (1.33 g, 91%) as a colorless oil, which slowly solidified to give a colorless solid. Mp: 106−7 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.73 (bs, 1H), 4.20 (m, 2H), 3.72 (s, 6H), 3.18 (t, 4H, *J* 12.8 Hz), 3.06 (1H), 2.54 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.8, 154.4, 80.3, 65.3, 52.1 (2C), 45.6 (2C), 39.2 (2C), 28.3 (3C). HRMS (ES): calcd for $C_{14}H_{23}NO_7 + Na^+$ 340.1372, found 340.1378.

General Procedure for Enzyme-Catalyzed Hydrolysis of 10. A mixture of **10** (15 mg) and an enzyme (5 mg) in phosphate buffer (0.2 M, pH 7.5, 1 mL) was stirred at room temperature. After total conversion (TLC control of starting material), the mixture was acidified to pH 2 with 2 N HCl and extracted with ethyl acetate. Drying (Na_2SO_4) and concentration of the combined organic phases provided **(3*R*)-** or **(3*S*)-11** as a colorless solid. Mp: 189−190 °C. ^{1}H NMR (200 MHz, acetone-d_6): δ 4.84 (bs, 1H), 4.25−4.05 (m, 2H), 3.66 (s, 3H), 3.00−3.25 (m, 2H), 2.54 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (50 MHz, acetone-d_6): δ 170.9, 170.1, 78.1, 64.9, 50.1, 45.1, 44.7, 39.2, 29.2. HRMS (ES): calcd for $C_{13}H_{21}NO_7 + Na^+$ 326.1216, found 326.1217.

(±)-(3,4-*cis*-4,5-*cis*)-1-Butyloxycarbonyl-4-hydroxypiperidine-3,5-dicarboxylic Acid 3-Methyl Ester [(±)-11]. To **10** (100 mg, 0.32 mmol) in THF/H_2O (1:1, 6 mL) was added NaOH (6.4 mg, 0.16 mmol). The reaction mixture was stirred for 20 min at room temperature and then neutralized with 2 N HCl. The THF in the mixture was removed through concentration. The remaining aqueous phase was washed with CH_2Cl_2 (2 × 10 mL), acidified to pH 2 with 2 N HCl and finally extracted with ethyl acetate (2 × 10 mL). The combined organic phases were dried ($MgSO_4$) and concentrated in vacuo to provide **(±)-11** (25 mg, 26%) as a colorless solid.

Analysis of the Enantiomerical Purity of 11. A sample of **11** was converted into the derivative **14** in the following manner. To a solution of **5** (10 mg, 0.033 mmol), benzylamine (0.1 mL) and Et_3N (0.1 mL) in CH_2Cl_2 (3 mL) was added HATU (20 mg, 0.053 mmol). After stirring for 10 min at room temperature, the reaction mixture was concentrated in vacuo. The residue was purified by chromatography (pentane/ethyl acetate 1:1, R_f 0.43) to provide **14** as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.22−7.10 (m, 5H), 6.60 (bs, 1H), 4.45 (1H), 4.40 (bs, 1H), 4.36 (d, 2H, *J* 5.6), 4.02 (m, 1H), 4.00 (dd, 1H, *J* 4.5, *J* 13.1 Hz), 3.67 (s, 3H), 3.24 (m, 1H), 3.18 (t, 1H, *J* 13.1 Hz), 2.40 (m, 1H), 2.35 (ddd, 1H, $J_{4,5}$ 2.4), 1.38 (s, 9H). HRMS (ES): calcd for $C_{20}H_{28}N_2O_6 + Na^+$ 415.1845, found 415.1839.

The optical purity of **14** was assessed by HPLC using a chiralpak OD column (i.d. 0.46 cm × 25 cm) and a 5:1 mixture of *n*-hexane/2-propanol as mobile phase. The flow rate was 0.5 mL/min, and peaks were detected at 260 nm. Racemic **14** gave identical sized peaks at 13.67 min [(*R*)-amide] and 15.59 min [(*S*)-amide].

(3*R*,4*S*)-*N*-*tert*-Butyloxycarbonyl-4-hydroxypiperidine-3-carboxylic Acid Methyl Ester (12). To a solution of **(3*R*)-11** (100 mg, 0.33 mmol, ee 78% obtained from PLE-catalyzed hydrolysis of **10**) and *N*-methylmorpholine (0.040 mL, 0.36 mmol) in THF (5 mL) was added ethyl chloroformate (0.035 mL, 0.037 mmol) at −15 °C. The obtained mixture was stirred for 5 min at the same temperature. Et_3N (0.100 mL, 0.72 mmol) and mercaptopyridine *N*-oxide (42 mg, 0.33 mmol) were then added, and the stirring was continued for 15 min at −15 °C. The precipatate of *N*-methylmorpholine hydrochloride was filtered and washed with THF.

To the yellow filtrate was added *tert*-butyl mercaptan (0.370 mL, 3.28 mmol). The obtained solution was irradiated at room temperature with a 150 W tungsten lamp for 20 min. The mixture was concentrated. The residue was taken up in H_2O (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). Drying (Na_2SO_4), concentration and chromatography (pentane/ethyl acetate 3:1, R_f 0.14) provided **12** (22 mg, 26%) as a colorless oil. $[\alpha]^{22}_D$ +37.6 (*c* 0.21, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.20 (m, 1H), 3.90 (m, 1H), 3.65 (s, 3H), 3.60 (m, 1H), 3.36 (t, 1H, *J* 10.4 Hz), 3.20 (ddd, 1H, *J* 12.0, *J* 3.2, *J* 13.6 Hz), 2.58 (ddd, 1H, *J* 4.8, *J* 2.4 Hz), 1.76 (dq, 1H, *J* 3.2, *J* 14.4 Hz), 1.60 (m, 1H), 1.40 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 80.0, 65.2, 52.2, 46.1, 42.0, 31.6, 28.6.

(3*R*,4*S*)-*N*-*tert*-Butyloxycarbonyl-4-hydroxypiperidine-3-carboxylic Acid Ethyl Ester (13). A mixture of **12** (22 mg, 0.085 mmol) and Na_2CO_3 (50 mg, 0.472 mmol) in EtOH (99%, 5 mL) was stirred for 5 h at room temperature. After reaction the mixture was concentrated in vacuo. The residue was taken into H_2O (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). Drying and concentration of the combined organic phases provided **13** (20 mg, 86%) as a colorless oil. $[\alpha]_D^{22}$ +31.4 (*c* 0.66, CH_2Cl_2). HRMS (ES): calcd for $C_{13}H_{23}NO_5 + Na^+$ 296.1474, found 296.1471.

(3*R*,4*S*,5*S*)-*N*-*tert*-Butyloxycarbonyl-4-hydroxypiperidine-3,5-dicarboxylic Acid 3-Methyl Ester [(3*R*)-11]. A mixture of **10** (8.46 g, 26.7 mmol) and lipase M (lipase *Mucor javanicus*, 2.0 g) in 350 mL of phosphate buffer (0.2 M, pH 7.5, 1 mL) was stirred for 3 days at room temperature. After reaction, the lipase was filtered off. The obtained solution was washed with CH_2Cl_2 (3 × 50 mL), acidified to pH 2 with 2 N HCl and then extracted with ethyl acetate (3 × 300 mL). Drying and concentration of the combined organic phases (ethyl acetate) provided **(3*R*)-11** (6.31 g, 95%, ee 99%) as a colorless solid. $[\alpha]^{22}_D$ +3.5 (*c* 1.3, MeOH).

(3*R*,4*S*,5*R*)-*N*-*tert*-Butyloxycarbonyl-4-hydroxy-5-hydroxymethyl-piperidine-3-carboxylic Acid Methyl Ester (15). To **(3*R*)-11** (3.0 g, 9.9 mmol) at −20 °C was added BH_3·THF (30 mL, 1 M in THF, 30.0 mmol) dropwise over 30 min. The obtained solution was stirred for 1 h at 0 °C. After reaction the excess BH_3·THF was destroyed by slow addition of H_2O. The mixture was extracted with diethyl ether (5 × 100 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to provide **15** (2.84 g, 99%) as a colorless foam. $[\alpha]^{22}_D$ +28.0 (*c* 1.6, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.50 (bs, 1H), 4.15 (m, 1H), 3.85 (m, 1H), 3.80−3.60 (m, 2H), 3.70 (s, 3H), 3.16 (m, 1H), 3.00 (m, 1H), 2.54 (m, 1H), 1.67 (m, 1H), 1.40 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.0, 154.8, 80.1, 67.0, 63.1, 52.1, 46.5, 41.6, 40.0, 29.7, 28.4. HRMS (ES): calcd for $C_{13}H_{23}NO_6 + Na^+$ 312.1423, found 312.1429.

(3*R*,4*S*,5*R*)-*N*-*tert*-Butoxycarbonyl-4-hydroxy-5-hydroxymethyl-4,5′-*O*-propylidene-piperidine-3-carboxylic Acid Methyl Ester (16). To a solution of **15** (2.84 g, 9.83 mmol) in 2,2-dimethoxypropane (100 mL) was added TsOH·H_2O (60 mg, 0.26 mmol) at room temperature. The resulting solution was stirred for 18 h at the same temperature and then washed with a saturated aqueous solution of $NaHCO_3$ (30 mL). The aqueous phase was extracted with ethyl acetate (2 × 30 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to provide **16** (3.01 g, 93%) as a colorless oil, which solidified slowly. Mp: 80−82 °C. $[\alpha]^{22}_D$ +16.8° (*c* 1.2, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.50 (bs, 1H), 4.05 (m, 1H), 4.02 (dd, 1H, *J* 3.0, *J* 13.0 Hz), 3.78 (m, 1H), 3.60 (s, 3H), 3.51 (d, 1H), 3.22 (t, 1H, *J* 12.8 Hz), 3.07 (t, 1H, *J* 12.8 Hz), 2.45 (ddd, 1H, *J* 3.4, *J* 3.0 Hz), 1.45 (m, 1H), 1.36 (s, 9H), 1.34/1.27 (2s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.3, 155.0, 99.0, 80.0, 66.5, 62.3, 51.9, 45.4 (2C), 34.0, 29.6, 28.6, 18.8. HRMS (ES): calcd for $C_{16}H_{27}NO_6 + Na^+$ 352.1736, found 352.1738.

(3*R*,4*S*,5*R*)-*N*-*tert*-Butoxycarbonyl-4-hydroxy-5-hydroxymethyl-4,5′-*O*-propylidene-piperidine-3-carboxylic Acid (17). To suspension of **16** (1.90 g, 5.8 mmol) in THF/H_2O (1:1, 20 mL) was added LiOH·H_2O (2.00 g, 47.6 mmol) at room temperature. The mixture was stirred for 4 h

at the same temperature. After reaction the mixture was neutralized with 2 N HCl. The THF was removed by concentration. The resulting aqueous phase was washed with CH_2Cl_2 (2 × 20 mL), acidified to pH 2 and extracted with ethyl acetate (3 × 50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to provide **17** (1.59 g, 87%) as a colorless solid. Mp: 135−8 °C. $[\alpha]^{22}_D$ +7.7° (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): *δ* 4.56 (bs, 1H), 4.08 (m, 1H), 4.05 (dd, 1H, *J* 3.0, *J* 13.0 Hz), 3.83 (m, 1H), 3.51 (d, 1H), 3.26 (t, 1H, *J* 12.5 Hz), 3.07 (t, 1H, *J* 12.5 Hz), 2.45 (ddd, 1H, *J* 3.5, *J* 3.1 Hz), 1.50 (m, 1H, H-5), 1.39 (s, 9H), 1.40/1.39 (2s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): *δ* 175.8, 155.2, 99.2, 80.4, 66.3, 62.3, 45.3 (2C), 33.7, 29.6, 28.6, 18.8. HRMS (ES): calcd for $C_{15}H_{25}NO_6$ + Na^+ 338.1580, found 338.1582.

(3*R*,4*S*,5*S*)-*N*-*tert*-Butoxycarbonyl-4-hydroxy-3-hydroxymethyl-5-phenyl-3′,4-*O*-isopropylidene-piperidine (18) and (3*R*,4*S*,5*R*)-*N*-*tert*-Butoxycarbonyl-4-hydroxy-3-hydroxymethyl-5-phenyl-3′,4-*O*-isopropylidene-piperidine (19). A mixture of **17** (56 mg, 0.18 mmol), $Pb(OAc)_4$ (200 mg, 0.45 mmol) and KOAc (500 mg, 5.09 mmol) in benzene (15 mL) was heated to reflux. After 5 h, the mixture was cooled, and the solid was removed by filtration. The solution was concentrated in vacuo, and the residue was purified by chromatography (pentane/ethyl acetate 10:1, R_f 0.17 for **18** and R_f 0.33 for **19**) to provide **18** (16 mg, 26%) and **19** (13 mg, 21%) as yellowish oils. Data for **18**. ^{1}H NMR (200 MHz, $CDCl_3$): *δ* 7.30−7.10 (m, 5H), 4.22 (t, 1H, *J* 2.4), 4.04 (dd, 1H, *J* 3.0, *J* 12.4 Hz), 4.04−3.74 (m, 2H), 3.50 (dd, 1H, *J* 1.1 Hz), 3.42 (t, 1H, *J* 12.8 Hz), 3.22 (t, 1H, *J* 12.8 Hz), 2.68 (ddd, 1H, *J* 4.1 Hz), 1.60 (m, 1H), 1.40 (s, 9H), 1.34/1.28 (s, 6H). HRMS (ES): calcd for $C_{20}H_{29}NO_4$ + Na^+ 370.1994, found 370.1997. HRMS (ES): calcd for $C_{20}H_{29}NO_4$ + Na^+ 370.1994, found 370.2003. Data for **19**. ^{1}H NMR (200 MHz, $CDCl_3$): *δ* 7.35−7.10 (m, 5H), 4.17 (t, 1H, *J* 2.4 Hz), 3.95 (dd, 1H, *J* 3.0, *J* 12.4 Hz), 4.30−3.60 (m, 2H), 3.50 (d, 1H), 3.49−3.24 (m, 2H), 2.80 (m, 1H), 1.62 (m, 1H), 1.40 (s, 9H), 1.42/1.35 (2s, 6H). HRMS (ES): calcd for $C_{20}H_{29}NO_4$ + Na^+ 370.1994, found 370.1997.

(3*R*,4*S*,5*S*)-*N*-*tert*-Butoxycarbonyl-4-hydroxy-5-hydroxymethyl-3-iodo-4,5′-*O*-isopropylidene-piperidine (20) and (3*R*,4*S*,5*R*)- *N*-*tert*-Butoxycarbonyl-4-hydroxy-5-hydroxymethyl-3-iodo-4,5′-*O*-isopropylidene-piperidine (21). A mixture of **17** (178 mg, 0.57 mmol), iodosobenzene diacetate (100 mg, 0.31 mmol) and I_2 (72 mg, 0.28 mmol) in CCl_4 (20 mL) was irradiated with Tungsten lamp (150 W) under reflux for 40 min. Another portion of iodosobenzene diacetate (100 mg, 0.31 mmol) and I_2 (72 mg, 0.28 mmol) was added. Irradiation was continued for 20 min. The reaction mixture was washed with diluted sodium thiosulfate. After drying (Na_2SO_4) and concentration the residue was separated by chromatography (pentane/ethyl acetate 11:1, R_f 0.16 for **20** and 0.27 for **21**) to provide **20** (101 mg, 45%) and **21** (83 mg, 37%) as brownish oils, which solidified slowly. Data for **20**. Mp: 81−82 °C. $[\alpha]^{22}_D$ +65.3 (*c* 1.2, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): *δ* 4.13 (m, 1H), 4.11 (bs, 1H), 4.10 (ddd, 1H, *J* 11.4, *J* 3.2), 4.02 (dd, 1H, *J* 2.7, *J* 12.3 Hz), 3.83 (m, 1H), 3.42 (d, 1H), 3.38−3.10 (m, 2H), 1.75 (m, 1H), 1.40 (s, 9H), 1.39 (s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): *δ* 100.0, 80.7, 69.1, 62.5, 41.7, 41.3, 29.7, 28.6, 18.8. HRMS (ES): calcd for $C_{14}H_{24}INO_4$ + Na^+ 420.0650, found 420.0648. Data for **21**. Mp: 59−61 °C. $[\alpha]^{22}_D$ −19.2 (*c* 1.2, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): *δ* 4.20 (bs, 1H), 4.10 (bs, 1H), 4.02 (dd, 1H, *J* 3.0, *J* 12.6 Hz), 4.20−3.87 (m, 1H), 3.56 (d, 1H), 3.35 (d, 1H), 3.22 (t, 1H, *J* 13.1 Hz), 2.16 (m, 1H), 1.43 (s, 9H), 1.36/1.33 (2s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): *δ* 155.0, 99.6, 80.2, 70.7, 62.9, 46.2, 42.0, 29.7, 28.6, 19.2. HRMS (ES): calcd for $C_{14}H_{24}INO_4$ + Na^+ 420.0650, found 420.0648.

(3*R*,4*S*,5*S*)-*N*-*tert*-Butoxycarbonyl-4-hydroxy-5-hydroxymethyl-4,5′-*O*-isopropylidene-3-trifluoroacetoxy-piperidine (22). To a solution of **20** (110 mg, 0.28 mmol) in ethyl acetate (10 mL) was added $AgOOCCF_3$ (500 mg, 2.26 mmol) at room temperature. The obtained mixture was stirred for 20 min at the same temperature, and then pentane (20 mL) was added. After the solid was filtered off, the solution was washed with H_2O (20 mL). The aqueous phase was extracted with pentane/ethyl acetate (3:1, 2 × 20 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to provide **22** (102 mg, 95%) as yellowish oil. $[\alpha]^{22}_D$ +42.5 (*c* 0.6, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): *δ* 4.72 (ddd, 1H, *J* 11.1, *J* 4.8, *J* 3.0), 4.45 (bs, 1H), 4.05 (m, 1H), 4.01 (dd, 1H, *J* 3.0, *J* 12.1 Hz), 3.81 (m, 1H), 3.58 (d, 1H), 3.30 (t, 1H, *J* 12.1), 3.16 (t, 1H, *J* 11.6 Hz), 1.58 (m, 1H), 1.39 (s, 9H), 1.35 (s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): *δ* 154.8, 123.0/117.3/111.7/106.0 (q), 99.3, 80.7, 73.6, 65.2, 61.8, 40.7 (bs), 33.8, 29.6, 28.5, 18.8.

(3*S*,4*S*,5*S*)-*N*-*tert*-Butoxycarbonyl-4-hydroxy-5-hydroxymethyl-4,5′-*O*-isopropylidene-3-trifluoroacetoxy-piperidine (23). To a solution of **21** (100 mg, 0.25 mmol) in ethyl acetate (10 mL) was added $AgOOCCF_3$ (400 mg, 1.81 mmol) at room temperature. The obtained mixture was stirred for 20 min at the same temperature, and then pentane (20 mL) was added. The mixture was poured into H_2O (20 mL), stirred for 10 min and then filtered. After separation of the phases, the aqueous phase was extracted with pentane/ethyl acetate (3:1, 2 × 20 mL). After drying and concentration of the combined organic phases, the residue was separated by chromatography to provide **23** (29 mg, 30%) and an unidentified compound (9 mg). Data for **23**. $[\alpha]^{22}_D$ +14.3 (*c* 0.6, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): *δ* 4.70 (bs, 1H), 4.44−3.64 (m, 2H), 4.14 (bs, 1H), 4.05 (dd, 1H, *J* 2.5, *J* 12.5 Hz), 3.58 (d, 1H), 3.22−2.90 (m, 2H), 1.58 (m, 1H), 1.40/1.35 (2s, 6H), 1.39 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): *δ* 99.4, 80.5, 73.2, 65.9, 61.9, 42.2, 40.5, 30.4, 29.7, 28.4, 19.0.

(3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-hydroxymethylpiperidine Hydrotrifluoroacetate (2). The mixture of **22** (102 mg, 0.27 mmol) in CF_3COOH/H_2O (2:1, 3 mL) was stirred for 5 min and then concentrated in vacuo to provide **2** (70 mg, quant) as yellowish solid. $[\alpha^{22}]_D$ +2.5 (*c* 1.0, H_2O). The ^{1}H NMR was identical to previously published spectra.[3,4]

Supporting Information Available: HPLC chromatogram of the **14** and **(±)-14**, ^{1}H and ^{13}C NMR spectra of the iodides **20** and **21** and the trifluoroacetates **22** and **23**, and ^{1}H and ^{13}C NMR spectral assignments for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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