The Synthesis of Diastereo- and Enantiomerically Pure *β*-Aminocyclopropanecarboxylic Acids

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The synthesis of diastereo- and enantiomerically pure *β*-aminocyclopropanecarboxylic acids (*β*-ACCs) is described. Starting from pyrrole, (*rac*)-**4** is readily obtained, which was kinetically resolved by enzymatic hydrolysis. Subsequent oxidation of (−)-**4** and deformylation gives rise to the *cis*-*β*-ACC derivative (*ent*)-**9**, while (+)-**10** was converted to the *trans*-*β*-ACC derivative **8**. Both **8** and (*ent*)-**9** and their benzyl esters **13** and **16**, being conformationally restricted *β*-alanine or *γ*-aminobutyric acid (GABA) derivatives, represent useful building blocks for peptides containing unnatural amino acids.

Introduction

α-Aminocyclopropanecarboxylic acids (α-ACCs) have been proved to be important because of their inherent biological activity[1,2] and because of their application as structural restricted building blocks for peptides.[3] A number of elegant strategies toward the synthesis of α-ACCs have emerged during recent years.[4,5]

β-Amino acids have also attracted considerable attention,[6] most spectacular as constituents of the newly emerging class of *β*-peptides.[7,8] There have been quite a few efforts toward the synthesis of *β*-aminocyclopropane carboxylic acids (*β*-ACCs),[9] being conformationally restricted *β*-alanine or *γ*-aminobutyric acid (GABA) derivatives, and their subsequent incorporation into peptides.[10] However, since *β*-ACC derivatives are vicinally substituted with donor−acceptor moieties,[11] they are extremely

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(1) Leading review: (a) Salaün, J.; Baird, M. S. *Curr. Med. Chem.* **1995**, *2*, 511−42.

(2) (a) O'Donell, M. J.; Bruder, W. A.; Eckrich, T. M.; Shullenberger, D. F.; Staten, G. S. *Synthesis* **1984**, 127−128. (b) Pirrung, M. C.; McGeehan, G. M. *Angew. Chem.* **1985**, *97*, 1074−1075. (c) Baldwin, J. E.; Adlington, R. M.; Lajoie, G. A.; Rawlings, B. J. *J. Chem. Soc., Chem. Commun.* **1985**, 1946. (d) Hill, R. K.; Prakash, S. R. *J. Am. Chem. Soc.* **1984**, *106*, 795. (e) Adams, D. O.; Yang, S. F. *Proc. Natl. Acad. Sci.* **1979**, *76*, 170. (f) Sakamura, S.; Ichihara, A.; Shiraishi, K.; Sato, H.; Nishiyama, K.; Sakai, R.; Furusaki, A.; Matsumoto, T. *J. Am. Chem. Soc.* **1977**, *99*, 636. (g) Mitchell, R. E. *Phytochemistry* **1985**, *24*, 1485. (h) Hoffman, N. E.; Yang, S. F.; Ichihara, A.; Sakamura, S. *Plant Physiol.* **1982**, *70*, 195. (i) Wakamiya, T.; Nakamoto, H.; Shiba, T. *Tetrahedron Lett.* **1984**, *25*, 4411. (j) Wakamiya, T.; Oda, Y.; Fujita, H.; Shiba, T. *Tetrahedron Lett.* **1986**, *27*, 2143. (k) Burroughs, L. F. *Nature* **1957**, *179*, 360.

(3) (a) Tsang, J. W.; Schmeid, M.; Nyfelter, R.; Goodman, M. J. *J. Med. Chem.* **1984**, *27*, 1663. (b) Mapelli, C.; Stammer, C. H.; Lok, S.; Mierke, D. F.; Goodman, M. *Int. J. Pept. Protein Res* **1988**, *32*, 484. (c) Zhu, Y. F.; Yamazaki, T.; Tsang, J. W.; Lok, S.; Goodman, M. *J. Org. Chem.* **1992**, *57*, 1074. (d) Ner, S. K.; Suckling, C. J.; Bell, A. R.; Wrigglesworth, R. *J. Chem. Soc., Chem. Commun.* **1987**, 480−482. (e) Kimura, H.; Stammer, C. H.; Shimohigashi, Y.; Cui, R. L.; Stewart, J. *J. Biochem. Biophys. Res. Commun* **1983**, *115*, 112. (f) Shimohigashi, Y.; Stammer, C. H.; Costa, T.; Voigtlander, v., P. F. *Int. J. Pept. Protein Res.* **1983**, *22*, 489. (g) Shimohigashi, Y.; Costa, T.; Nitz, T. J.; Chen, H. C.; Stammer, C. H. *Biochem. Biophys. Res. Commun.* **1984**, *121*, 966. (h) Mapelli, C.; Kimura, H.; Stammer, C. H. *Int. J. Pept. Protein Res.* **1986**, *28*, 347. (i) Ahmad, S.; Phillips, R. S.; Stammer, C. H. *J. Med. Chem.* **1992**, *35*, 1410. (j) Burgess, K.; Ho, K.-K.; Pettitt, B. M. *J. Am. Chem. Soc.* **1995**, *117*, 54−65. (k) Burgess, K.; Ho, K.-K. *J. Am. Chem. Soc.* **1994**, *116*, 799−800. (l) Malin, D. H.; Payza, K.; Lake, J. R.; Corriere, L. S.; Benson, T. M.; Smith, D. A.; Baugher, R. K.; Ho, K.-K.; Burgess, K. *Peptides* **1993**, *14*, 47. (m) Malin, D. H.; Lake, J. R.; Ho, K.-K.; Corriere, L. S.; Garber, T. M.; Waller, M.; Benson, T.; Smith, D. A.; Luu, T.-A.; Burgess, K. *Peptides* **1993**, *14*, 731. (n) Moye-Sherman, D.; Jin, S.; Li, S.; Welch, M. B.; Reibenspies, J.; Burgess, K. *Chem. Eur. J.* **1999**, *5*, 2730−39.

(4) Leading reviews: (a) Doyle, M. P.; Protopopova, M. N. *Tetrahedron* **1998**, *54*, 7919−7946. (b) Burgess, K.; Ho, K.-K.; Moyesherman, D. *Synlett* **1994**, 575−583. (c) Stammer, C. H. *Tetrahedron* **1990**, *46*, 2231.

(5) (a) Burgess, K.; Ho, K.-K. *J. Org. Chem.* **1992**, *57*, 5931−36. (b) Aufranc, P.; Ollivier, J.; Stolle, A.; Bremer, C.; Es-Sayed, M.; de Meijere, A.; Salaün, J. *Tetrahedron Lett.* **1993**, *34*, 4193−4196. (c) Dorizon, P.; Su, G.; Ludvig, G.; Nikitina, L.; Paugam, R.; Ollivier, J.; Salaün, J. *J. Org. Chem.* **1999**, *64*, 4712−24. (d) Es−Sayed, M.; Gratkowski, C.; Krass, N.; Meyers, A. I.; de Meijere, A. *Synlett* **1992**, 962−964. (e) de Meijere, A.; Ernst, K.; Zuck, B.; Brandl, M.; Kozhushkov, S. I.; Tamm, M.; Yufit, D. S.; Howard, J. A. K.; Labahn, T. *Eur. J. Org. Chem.* **1999**, *3105*, 5−3115.

(6) (a) Juaristi, E., Ed. *Synthesis of β-amino acids*; Wiley-VCH: New York, 1997. (b) Cole, D. C. *Tetrahedron* **1994**, *50*, 9517−9582. (c) Palomo, C.; Oiarbide, M.; González-Rego, M. C.; Sharma, A. K.; García, J. M.; González, A.; Landa, C.; Linden, A. *Angew. Chem.* **2000**, *112*, 1105−1107. (d) Tang, T. P.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 12−13. (e) Miyabe, H.; Fujii, K.; Naito, T. *Org. Lett.* **1999**, *1*, 569−572. (f) Marcotte, S.; Pannecoucke, X.; Feasson, C.; Quirion, J.-C. *J. Org. Chem.* **1999**, *64*, 8461−64. (g) Nocioni, A. M.; Papa, C.; Tomasini, C. *Tetrahedron Lett.* **1999**, *40*, 8453−56. (h) Davies, S. G.; Ichihara, O. *Tetrahedron Lett.* **1999**, *40*, 9313−16. (i) Enders, D.; Wahl, H.; Bettray, W. *Angew. Chem.* **1995**, *107*, 527−529. (j) Es-Sayed, M.; Gratkowski, C.; Krass, N.; Meyers, A. I.; Meijere de, A. *Tetrahedron Lett.* **1993**, *34*, 289−292. (k) Jefford, C. W.; Wang, J. *Tetrahedron Lett.* **1993**, *34*, 1111−14. (m) Juaristi, E.; Quintana, D.; Escalante, J. *Aldrichim. Acta* **1994**, *27*, 3−11. (n) Burgess, K.; Liu, L. T.; Pal, B. *J. Org. Chem.* **1993**, *58*, 4758−4763. (o) Lombardi, A.; Saviano, M.; Nastri, F.; Maglio, O.; Mazzeo, M.; Isernia, C.; Paolillo, L.; Pavone, V. *Biopolymers* **1996**, *38*, 693. (p) Pavone, V.; Lombardi, A.; Yang, X.; Pedone, C.; Blasio, B. D. *Biopolymers* **1990**, *30*, 189−196. (q) Blasio, B. D.; Lombardi, A.; Yang, X.; Pedone, C.; Pavone, V. *Biopolymers* **1991**, *31*, 1181−1188. (r) Pavone, V.; Lombardi, A.; D'Auria, G.; Saviano, M.; Nastri, F.; Paolillo, L.; Blasio, B. D.; Pedone, C. *Biopolymers* **1992**, *32*, 173−183. (s) Pavone, V.; Lombardi, A.; Maggi, C. A.; Quartara, L.; Pedone, C. *J. Pept. Sci.* **1995**, *1*, 236−240. (t) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 1054−1062.

(7) Leading reviews: (a) Seebach, D.; Matthews, J. L. *Chem. Commun.* **1997**, 2015−2022. (b) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173.

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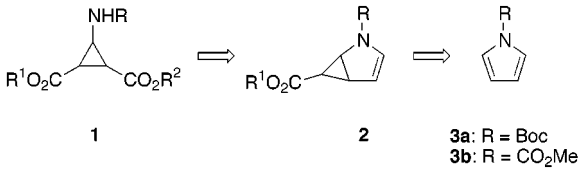
prone toward ring opening. Special requirements are therefore necessary for their synthesis and subsequent use as building blocks.[12]

We report here a synthetic strategy for the preparation of diastereo- and enantiomerically pure 3-aminocyclopropane-1,2-dicarboxylic acids starting from *N*-Boc-pyrrole (**3a**) that allows the subsequent incorporation of both, the *cis*- and the *trans*-β-ACC structure into peptides. The key intermediate **2** may be transformed into **1** with chemical differentiation of the ester groups to allow their individual use in subsequent synthetic applications.

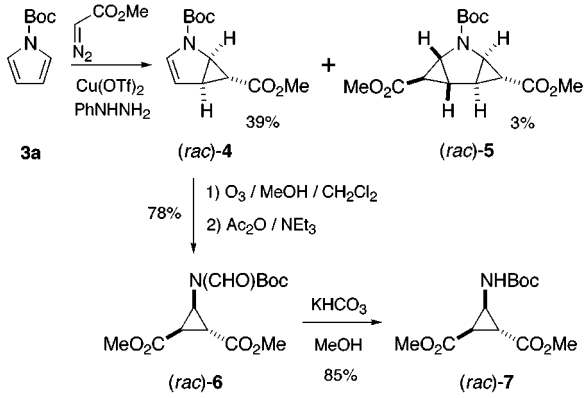


1 **2** **3a**: R = Boc; **3b**: R = CO_2Me

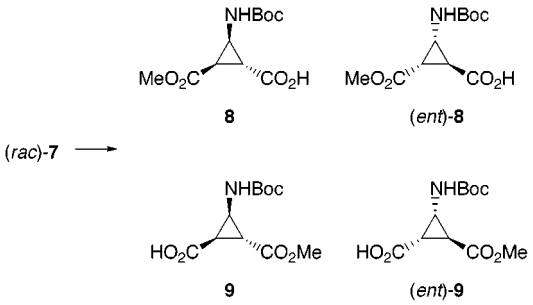
Results and Discussion

Synthesis of Racemic β-Aminocyclopropanecarboxylic Acid Derivatives. The cyclopropanation of *N*-carbomethoxypyrrole (**3b**) with methyl diazoacetate has been reported by Fowler using copper(I) bromide as the catalyst at 80 °C to yield **2** in 17% yield.[13] We were able to improve this reaction by using catalytic amounts of copper(II) triflate, activated by phenylhydrazine. This way, the cyclopropanation of **3a** proceeds smoothly at room temperature, yielding (*rac*)-**4** in 39% yield along with the 2-fold cyclopropanated adduct (*rac*)-**5** (3%) and recovered starting material (36%). (*rac*)-**4** is obtained as a single diastereomer, having the ester group oriented at the convex face of the bicyclic structure. However, we have been unable to find a chiral catalyst so far that would achieve the synthesis of optical active **4** with preparative useful enantioselectivities. Therefore, we investigated the possibility of an enzymatic resolution of (*rac*)-**4** as well as of the aminocyclopropane dimethylester (*rac*)-**7**, which is conveniently obtained from (*rac*)-**4** by ozonolysis followed by deprotection of the *N*-formyl group (Scheme 1).

Scheme 1



Scheme 2



Kinetic Enzymatic Resolution of (*rac*)-7. The diester (*rac*)-**7** contains two nonequivalent ester functions; therefore, the hydrolysis of a single ester bond of (*rac*)-**7** could lead to four different stereoisomers (Scheme 2). Consequently, the synthesis of one enantiomer is only possible if the biocatalyst shows high enantioselectivity and regioselectivity at the same time.

The screening for enzymes capable of the hydrolysis of (*rac*)-**7** was carried out either in a buffered aqueous suspension or in a two-phase system consisting of buffer/toluene. From the enzymes screened (Table 1) only pig liver esterase (PLE, E-1 and E-2) and lipase from *Mucor miehei* (L-9) were able to hydrolyze the substrate in significant amounts while the other enzymes showed only low activity or were inactive at all.

The nearly complete conversion observed in the cases of L-9 and PLE indicated that only poor enantiospecificity had occurred. Furthermore, the product isolated from a preparative scale using PLE A as the biocatalyst showed in addition poor regioselectivity as determined by ^{1}H NMR spectroscopy. The data revealed that approximately 40% of the *cis*-amino acid **9** and 60% of the *trans*-amino acid **8** had been produced, suggesting that the resolution

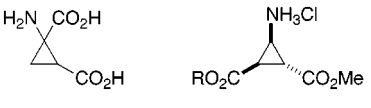
(8) (a) Apella, D.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071−72. (b) Huck, B. R.; Langenhan, J. M.; Gellman, S. H. *Org. Lett.* **1999**, *1*, 1717−20. (c) Applequist, J.; Bode, K. A.; Appella, D. A.; Christianson, L. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4891. (d) Krauthäuser, S.; Christianson, L. A.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 11719. (e) Appella, D. H.; Christianson, L. A.; Klein, D.; Powell, D. R.; Huang, X.; Barchi, J. J.; Gellman, S. H. *Nature* **1997**, *387*, 381. (f) Seebach, D.; Abele, S.; Gademann, K.; Jaun, B. *Angew. Chem.* **1999**, *111*, 1700−02. (g) Gademann, K.; Ernst, M.; Hoyer, D.; Seebach, D. *Angew. Chem.* **1999**, *111*, 1302−1304. (h) Seebach, D.; Matthews, J. L. *Helv. Chim. Acta* **1997**, *80*, 173−182. (i) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B. *Helvetica Chimica* **1996**, *79*, 913−932. (j) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Hev. Chim. Acta* **1996**, *1996*, 913−41.

(9) (a) Cannon, J. G.; Garst, J. E. *J. Org. Chem.* **1975**, *40*, 182−184. (b) Shroff, C. C.; Stewart, W. S.; Uhm, S. J.; Wheeler, J. W. *J. Org. Chem.* **1971**, *22*, 3356−3361. (c) Paulini, K.; Reissig, H.-U. *Liebigs Ann. Chem.* **1991**, 455−461. (d) Bubert, C.; Voigt, J.; Biasetton, S.; Reiser, O. *Synlett* **1994**, 675−677. (e) Vilsmaier, E. In *The Chemistry of the Cyclopropyl Group*; Rappoport, Z., Ed.; VCH−Wiley: New York, 1987; p 1341.

(10) (a) Paulini, K.; Reissig, H.-U. *Liebigs Ann. Chem.* **1994**, 549−554. (b) Beck-Sickinger, A. G.; Hoffmann, E.; Paulini, K.; Reissig, H.-U.; Willim, K.-D.; Wieland, H. A.; Jung, G. *Biochem. Soc. Trans.* **1994**, *22*, 145−149. (c) Hibbs, D. E.; Hursthouse, M. B.; Jones, I. G.; Jones, W.; Malik, K. M. A.; North, M. *Tetrahedron* **1997**, *53*, 17417−24. (d) Voigt, J.; Noltemeyer, M.; Reiser, O. *Synlett* **1997**, 202−204. (e) Bubert, C.; Cabrele, C.; Reiser, O. *Synlett* **1997**, 827−29.

(11) Reissig, H. U. *Top. Curr. Chem.* **1988**, *144*, 73.

(12) A racemic synthesis of *E*- and *Z*-cyclo-Asp without occurrence of ring opening was reported: Kraus, G. A.; Kim, H.; Thomas, P. J.; Metzler, D. E.; Metzler, C. M.; Taylor, J. E. *Synth. Commun.* **1990**, *20*, 2667. We have never been able to synthesize a β-ACC derivative with an unprotected amino group. The closest we ever got has been the synthesis of the diester **A** (R = Bn or Me),[10e] but already the corresponding acid (R = H) proved to be unstable and could also not be observed in situ by NMR spectroscopy.



cyclo-Asp **A**

(13) Tanny, S. R.; Grossman, J.; Fowler, F. W. *J. Am. Chem. Soc.* **1972**, *94*, 6495−6501.

Table 1. Screening for Esterases and Lipases for the Hydrolysis of (*rac*)-7

enzyme[a]	screening conditions[b]	investigated period of time (h)	conversion[c]
PLE A	suspension	120	nearly complete
E-1	suspension	24	nearly complete
E-2	suspension	24	>50%
PPL	suspension	120	traces
L-1	water/toluene	165	no conversion
L-2	water/toluene	165	no conversion
L-3	water/toluene	165	traces
L-5	water/toluene	165	no conversion
L-6	water/toluene	165	traces
L-7	water/toluene	165	no conversion
L-8	water/toluene	165	traces
L-9	water/toluene	165	nearly complete

[a] For enzyme abbreviations see Table 6 in the Supporting Information. [b] See the Experimental Section. [c] Determined by qualitative TLC.

step at the level of the cyclopropane diester (*rac*)-**7** is impossible with the enzymes available to us. The enantioselectivity of the reaction products was therefore not investigated in detail.

The lack of discrimination between the *cis*- and *trans*-ester group for (*rac*)-**7** has been surprising in light of PLE-catalyzed hydrolyses of a variety of cyclopropane dicarboxylic esters being described in the literature[14] and the models which have consequently been developed for such processes.[15] It should be kept in mind that commercially available PLE consists of a mixture of isoenzymes which can behave more or less differently in regard to substrate- and enantiospecificity.[14] For example, E-1 and E-2 used in our screening experiments represent different fractions of the isoenzymes of PLE.

Kinetic Enzymatic Resolution of (*rac*)-4. We focused next on the kinetic enzymatic resolution of the bicyclic adduct (*rac*)-**4** which is used as a direct precursor for β-ACC derivatives. From the enzymes screened (Table 2) pig liver esterase (PLE A, E−1 and E−2) and lipases (L-1, L-2, L-6) were shown to carry out the desired transformation with significant conversion while other enzymes showed only low activity (L-9) or were inactive at all.

Encouragingly, L-1, L-6, and L-9 indeed were capable of hydrolyzing (*rac*)-**4** without complete conversion even after 10 d of reaction time. Furthermore, although the reaction with L-2 was complete, the rate decreased considerably during the course of the reaction in difference to the esterases, suggesting that resolution with L-2 might also be possible.

Next, kinetic measurements were carried out combined with determination of the enantioselectivities being obtained with the enzymes PLE A, L-1, L-2, and L-6 in the hydrolysis of (*rac*)-**4**. L-9 was not considered for further experiments since its activity was too low in order to be useful for preparative transformations. For each enzyme the change of substrate and product concentration with time was recorded (Figures 1a−4a) and the enantiomeric excess of the substrate **4** was plotted versus the conversion (Figures 1b−4b). In the latter diagrams, the solid lines represent simulations for the determination of the enantiomeric ratio (E value) according to the model described by Chen et al.[16] and fit well the experimental data.

Table 2. Screening for Esterases and Lipases with Activity for the Hydrolysis of (*rac*)-4

enzyme[a]	screening conditions[b]	investigated period of time (h)	conversion[c]
PLE A	suspension	24	complete
E-1	suspension	24	complete
E-2	suspension	24	nearly complete
L-1	water/toluene	240	∼50%
L-2	water/toluene	72	complete
L-3	water/toluene	240	no conversion
L-5	water/toluene	240	no conversion
L-6	water/toluene	240	∼50%
L-7	water/toluene	240	no conversion
L-8	water/toluene	240	no conversion
L-9	water/toluene	240	≪50%
A2	water/toluene	187	no conversion
A4	water/toluene	187	traces
A6	water/toluene	187	traces
A8	water/toluene	187	no conversion
A9	water/toluene	187	traces
A10	water/toluene	187	no conversion
A11	water/toluene	187	no conversion

[a] For enzyme abbreviations see Table 6 in the Supporting Information. [b] See the Experimental Section. [c] Determined by qualitative TLC.

Table 3. Activity and Selectivity of Enzymes for the Kinetic Resolution of (*rac*)-4

enzyme[a]	reaction conditions[b]	activity[c] (μmol/min × mg)	preferentially hydrolyzed enantiomer	enantiomeric ratio E[d]
PLE A	A	0.46	(+)-**4**	1.7
L-1	A	6.3×10^{-3}	n.d.	n.d.
	B	4.0×10^{-5}	(−)-**4**	3.5
L-2	A	0.33	(+)-**4**	4.6
	B	6.9×10^{-3}	(+)-**4**	36.0
L-6	A	6.9×10^{-3}	n.d.	n.d.
	B	3.8×10^{-5}	(+)-**4**	8.5

[a] For enzyme abbreviations see Table 6 in the Supporting Information. [b] A: 0.1 M phosphate buffer pH 7.5, 25 °C, automatic buret. B: toluene/0.1 M phosphate buffer pH 7.4, ambient temperature. [c] Specific enzyme activity (initial activity, total conversion of both enantiomers of (*rac*)-**4**). [d] According to Chen et al.[16]

On the basis of these studies, the determination of the activity and selectivity of the enzymes tested for the kinetic resolution of (*rac*)-**4** became possible (Table 3). PLE A shows the highest activity, but also the lowest enantioselectivity. From the three lipases investigated L-2 displayed the highest activity and, what is more important, also the highest enantioselectivity. Interestingly, L-1 was found to hydrolyze (−)-**4** preferentially, whereas (+)-**4** was hydrolyzed faster by all the other enzymes. The specific activity of the lipases in aqueous buffer is approximately 2 orders of magnitude higher than in a biphasic system consisting of toluene and buffer.

Lipase L-2 was chosen for further optimization of the reaction conditions because of its most promising characteristics. The other enzymes revealed major drawbacks either in low activity (L-1 and L-6) and/or enantioselectivity (PLE A and L-1).

To minimize the amount of enzyme used in the screening experiments and to enhance the enantioselec-

(14) Gais, H. J. Hydrolysis of Carboxylic acid esters. In *Enzyme Catalysis in Organic Synthesis*; Drauz, K. H., Waldmann, H., Eds.; VCH−Wiley: Weinheim, 1995; Vol. 1, Chapter B.1.1.1.1, pp 178−195.

(15) (a) Toone, E. J.; Werth, M. J.; Jones, J. B. *J. Am. Chem. Chem. Soc.* **1990**, *112*, 4946−4952. (b) Walser, P.; Renold, P.; N'Goka, V.; Hosseinzadeh, F.; Tamm, C. *Helv. Chim. Acta* **1991**, *74*, 1941−1952. (c) Provencher, L.; Jones, J. B. *J. Org. Chem.* **1994**, *59*, 2729−2732.

(16) Chen, C. S.; Fujimoto, Y.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299.

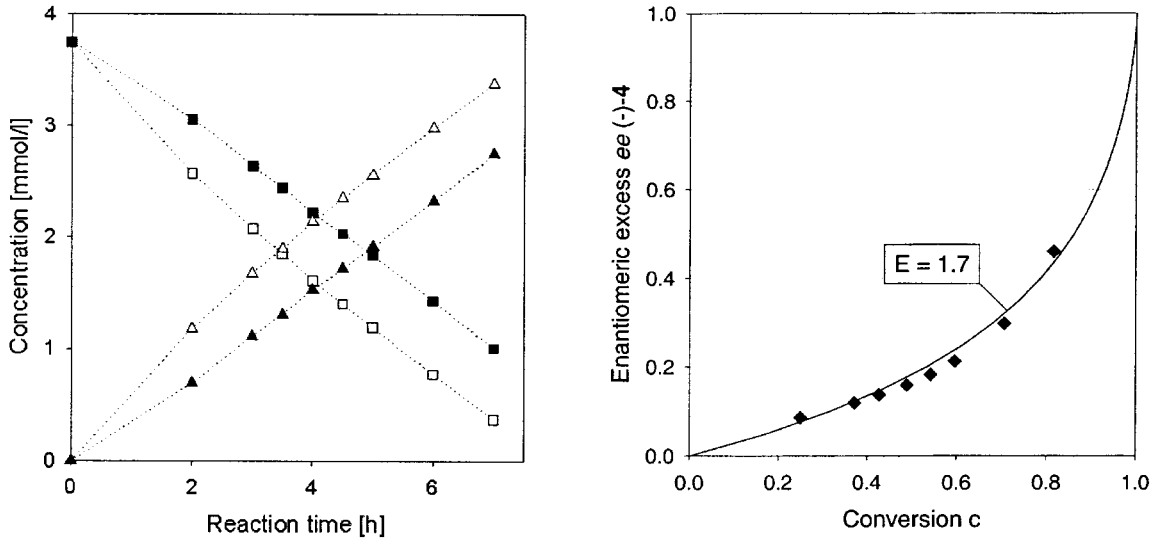


Figure 1. PLE A catalyzed kinetic resolution of (*rac*)-**4** (screening). (a) Time course of the concentrations of substrate and product enantiomers. (b) Enantiomeric excess of (−)-**4** (ee(*S*)) versus conversion. (□ (+)-**4**; ■ (−)-**4**; △ (+)-**10**; ● (−)-**10**).

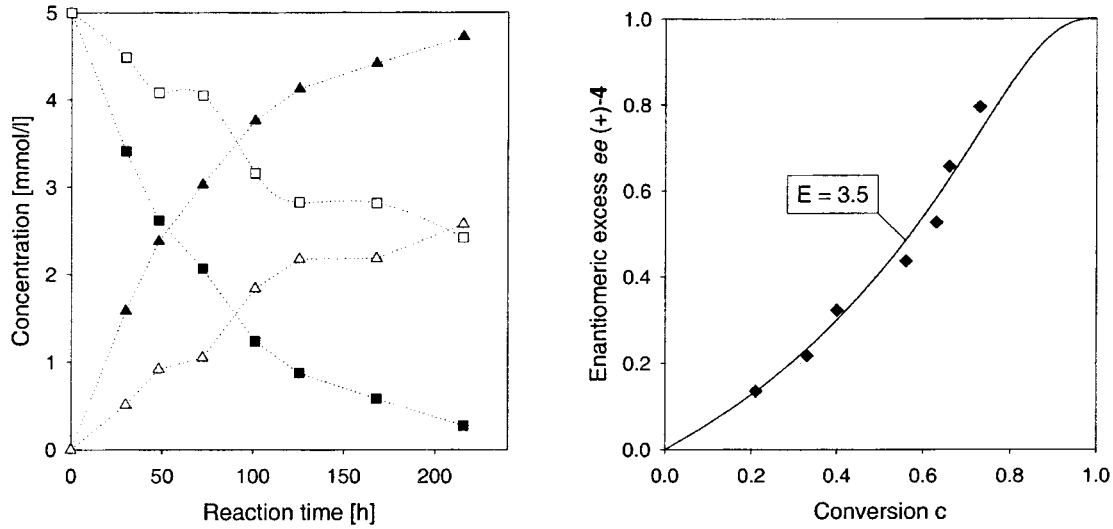


Figure 2. Lipase L-1 catalyzed kinetic resolution of (*rac*)-**4** (screening). (a) Time course of the concentrations of substrate and product enantiomers. (b) Enantiomeric excess of (+)-**4** (ee(*S*)) versus conversion. (□ (+)-**4**; ■ (−)-**4**; △ (+)-**10**; ● (−)-**10**).

tivity, the reaction conditions (cosolvent, amount of enzyme, addition of Triton X-100; for details see Table 4 in the Supporting Information) were varied. All attempts failed to enhance the selectivity factor E above 36, which was initially found. However, the optimization experiments revealed two important developments. The amount of enzyme needed for the transformation could be reduced by a factor of approximately 100 (from 13 to 0.12 g enzyme per gram substrate). As a minor drawback the selectivity factor E dropped from 36 to 13.5. On the other hand the addition of Triton X-100 simplified homogeneous sampling by stabilizing the reaction emulsion leaving the enantioselectivity of the enzyme almost unchanged.

Using the optimized reaction conditions, the kinetic resolution (*rac*)-**4** could be carried out on a gram scale. For example, starting with 1 g of (*rac*)-**4** to the point of complete depletion of (+)-**4**, 335 mg of (−)-**4** (33%, 99.6% ee) and 462 mg (49%, 43.3% ee) of (+)-**10** were isolated. From these data, the selectivity factor calculated (E = 13.4) is in excellent agreement with the one determined during the optimization process (E = 13.5, cf. Figure 5).

It was not possible to obtain enantiopure acid (+)-**10** in preparative useful amounts at the E value achieved during the synthesis of enantiopure (−)-**4**. Therefore, the reaction conditions were optimized further. Using cyclohexane as a cosolvent, the pH was varied between 5.0 and 8.0. Below and above these pH values, substrate and product were unstable. The optimum of the enantioselectivity is found at pH 5.0, whereas the activity was highest at alkaline pH. For comparison, in Figure 5b the enantiomeric excess of **10** is shown versus the conversion for the initial optimization pH 7.4 and the optimal pH of 5.0.

Consequently, using lipase L-2 and the optimized reaction conditions (*cf.* experimental part and Table 5, Supporting Information) 303 mg of (+)-**10** (30% yield, 91% ee) was obtained by kinetic resolution of 1.067 g (*rac*)-**4**. Subsequent recrystallization yielded (+)-**10** in enantiomerically pure form (>96% ee).

Synthesis of *cis*- and *trans*-β-Aminocyclopropanecarboxylic Acid Derivatives. Having (−)-**4** in enantiomerically pure form in hand, it can be readily transformed into the *cis*-β-ACC derivative **13** (Scheme 3):

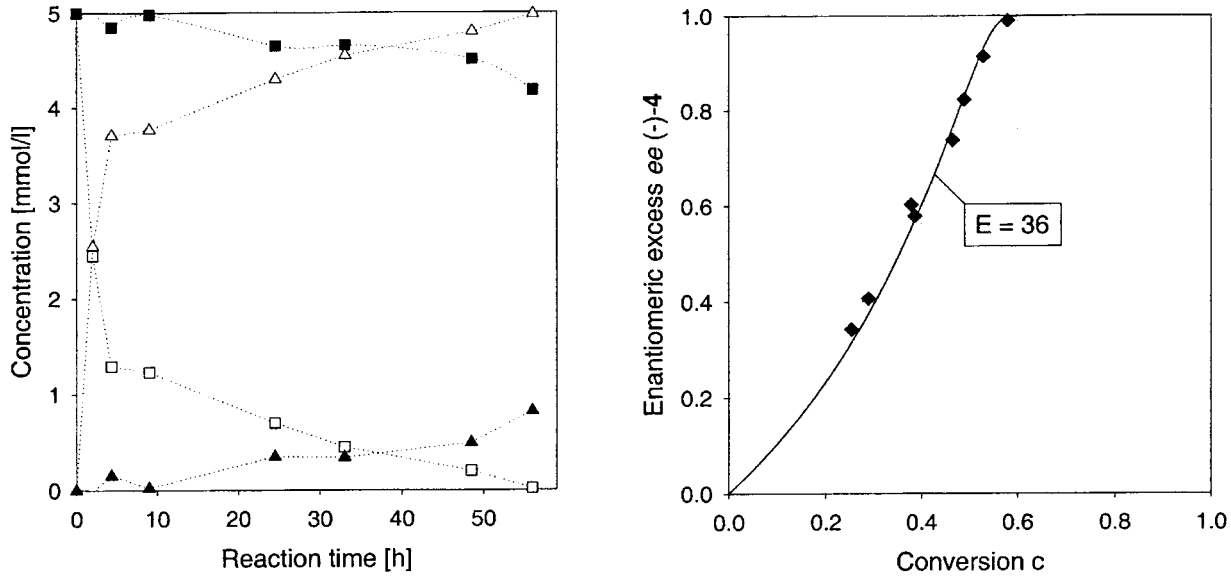


Figure 3. Lipase L-2 catalyzed kinetic resolution of (*rac*)-**4** (screening). (a) Time course of the concentrations of substrate and product enantiomers. (b) Enantiomeric excess of (−)-**4** (ee(*S*)) versus conversion. (□ (+)-**4**; ■ (−)-**4**; △ (+)-**10**; ▲ (−)-**10**).

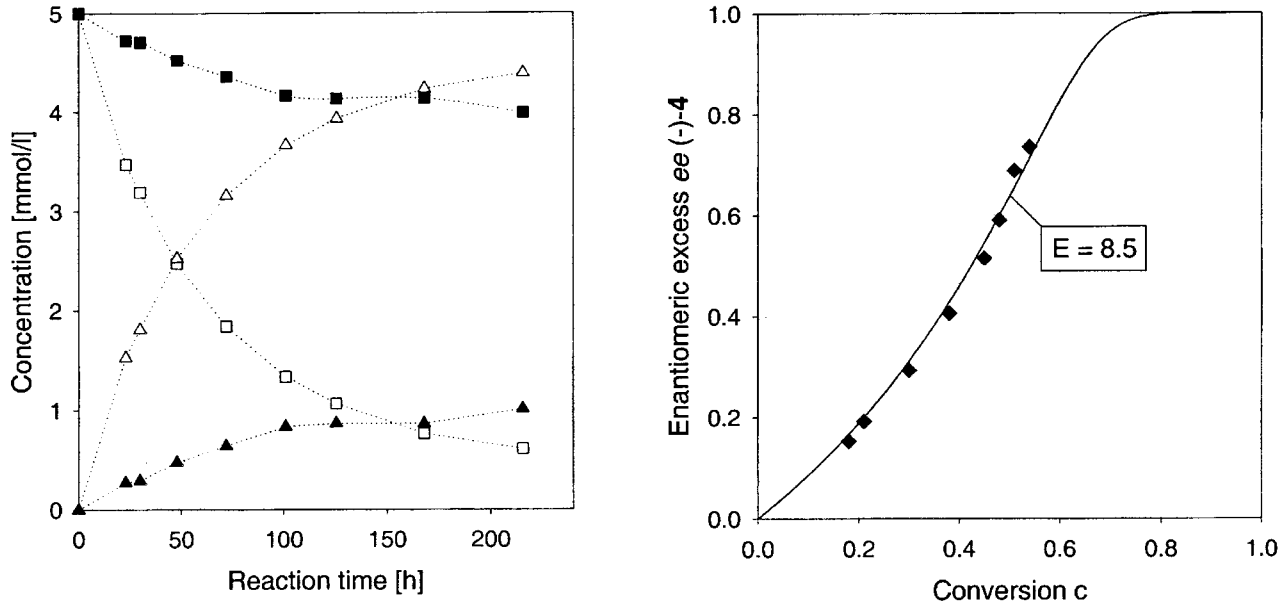


Figure 4. Lipase L-6 catalyzed kinetic resolution of (*rac*)-**4** (screening). (a) Time course of the concentrations of substrate and product enantiomers. (b) Enantiomeric excess of (−)-**4** (ee(*S*)) versus conversion. (□ (+)-**4**; ■ (−)-**4**; △ (+)-**10**; ▲ (−)-**10**).

Ozonolysis of (−)-**4** followed by reductive workup with dimethyl sulfide gave rise to the amino aldehyde **11**. Due to the two electron withdrawing protecting groups at nitrogen, **11** is perfectly stable and has already proved in racemic form to be a valuable building block for further synthetic transformations.[17]

Oxidation of **11** with $NaClO_2/H_2O_2$ proceeded smoothly to the carboxylic acid **12**, followed by deprotection of the *N*-formyl group to give rise to (*ent*)-**9**. Although a variety of bases are able to readily facilitate this deformylation, special care is necessary in this step; e.g., deprotection with potassium bicarbonate proceeded with good chemical yield (85%) but led to complete epimerization on the amino substituted carbon atom. Fortunately, deprotection with amines such as piperidine, morpholine or especially 2-diethylaminoethylamine (DEAEA)[18] took place without any detectable scrambling on any of the stereocenters in high yields (87−95%). Finally, esterification with benzyl bromide gave rise to **13**, which can be incorporated into peptides as the *cis*-*β*-ACC structure as has been demonstrated by us before.[10e] Moreover, (*ent*)-**9** was converted to the amide **14**, from which a X-ray structure was obtained in order to unambiguously establish the absolute stereochemistry of all *β*-ACC derivatives described here.

Starting from (+)-**10** the *β*-ACC derivative **16** can be readily obtained (Scheme 4) by similar transformations as depicted for the synthesis of **13**. Thus, ozonolysis and workup with Ac_2O/NEt_3 resulted in **15** which was directly

(17) (a) Bubert, C.; Reiser, O. *Tetrahedron Lett.* **1997**, *38*, 4985−88. (b) Böhm, C.; Schinnerl, M.; Bubert, C.; Zabel, M.; Labahn, T., Parisini, E.; Reiser, O. *Eur. J. Org. Chem.* **2000**, 2955−65.

(18) Grehn, L.; Gunnarson, K.; Ragnarsson, U. *Chem. Commun.* **1985**, 1317−18.

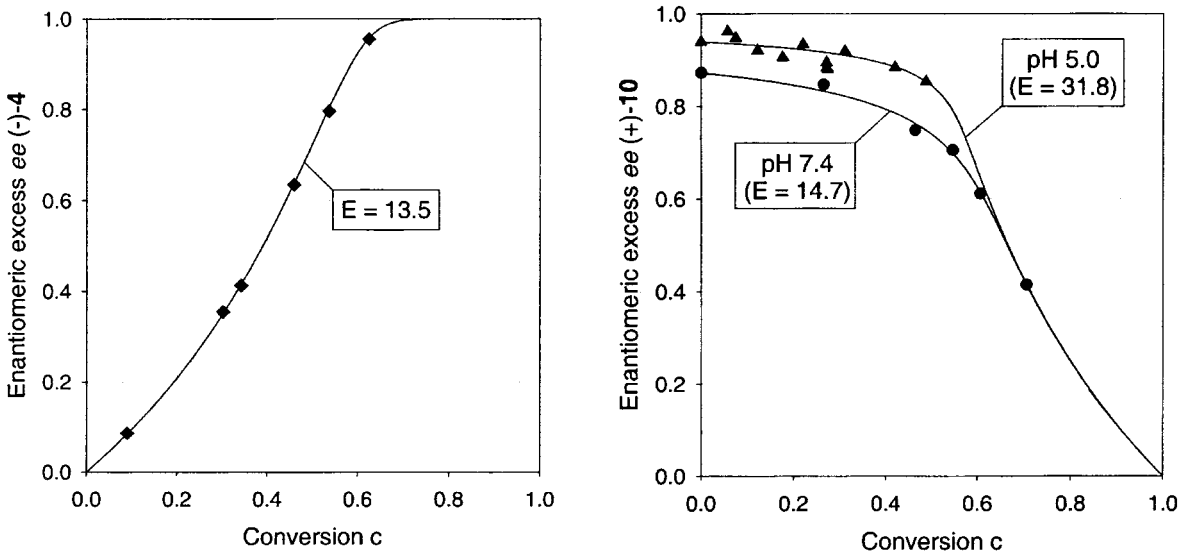
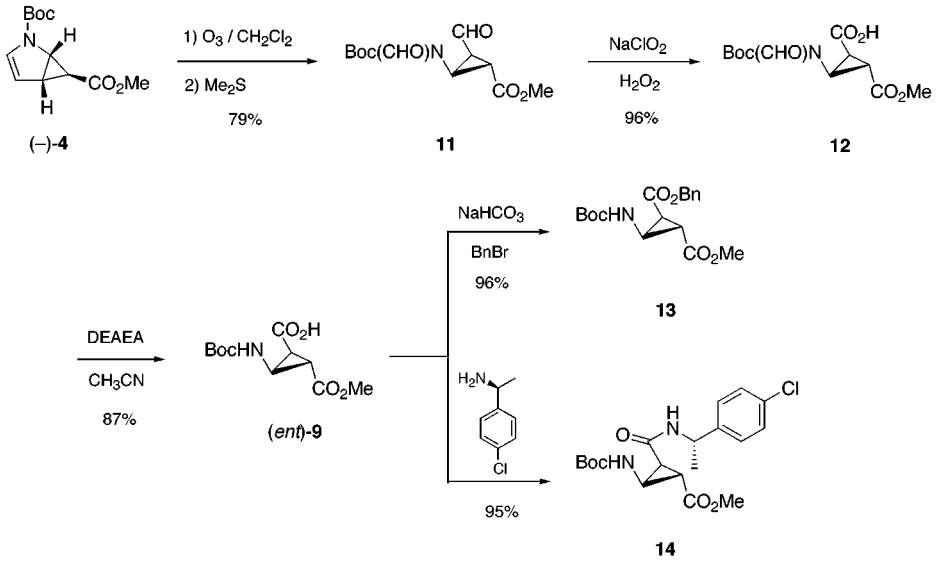
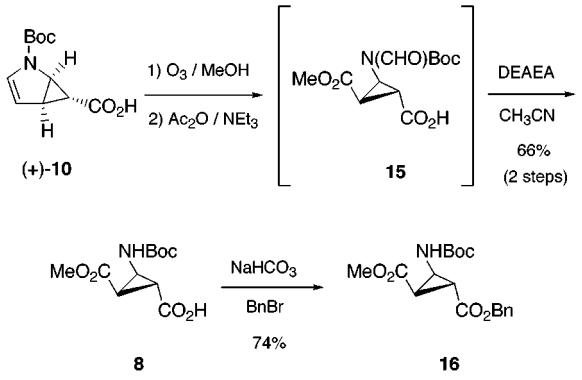


Figure 5. Lipase L-2 catalyzed kinetic resolution of (*rac*)-**4** (preparative scale). (a) Enantiomeric excess of (−)-**4** (ee(*S*)) versus conversion. (b) Enantiomeric excess versus conversion for the product of the reaction 10 before (pH 7.4) and after optimization (pH 5.0).

Scheme 3



Scheme 4



deformylated with DEAEA to yield **8** (66% from (+)-**10**). The sequence was completed with the synthesis of the benzyl ester **16**. Both, **8** or **16** can be used as a starting point for incorporation of the *trans*-*β*-ACC structure into peptides complementing (*ent*)-**9** and **13** in which the *cis*-*β*-ACC structure can be utilized.[10e]

In summary, a short synthesis to diastereo- and enantiomerically pure *β*-ACC derivatives **8**, (*ent*)-**9**, **13**, and **16** has been developed which are suitable building blocks for peptides. Especially, the incorporation of the *cis*-*β*-ACC structure seems to be interesting as a turn mimic, and first results in the synthesis of NPY-mimics having (*ent*)-**9** incorporated show promising selectivites to specific Neuropeptide Y receptors.[19]

Experimental Section

General Methods. GC analysis was performed on a Heptakis-(2,3-di-*O*-methyl-6-*O*-thexyldimethylsilyl)-*β*-cyclodextrine capillary column (15 m × 0.25 mm) prepared and supplied by Prof. König (Institute of Organic Chemistry, University of Hamburg, Germany). Helium was used as carrier gas at a pressure of 40 kPa. Temperature program: 100 °C, 4 min isotherm, 3 °C/min to 128 °C, 13 min isotherm, 6 °C/min to 180 °C, 10 min isotherm, 6 °C/min to 100 °C. Retention times: (+)-**4**: 22.258 min, (−)-**4**: 23.471 min, α = 1.05.

(19) Beumer, R.; Cabrele, C.; Beck-Sickinger, A.; Reiser, O. Unpublished results.

Chiral-HPLC. The separation of the enantiomers of **4**, **7** and **10** was accomplished using a ET 200/4 Nucleodex β-PM column (Macherey-Nagel, Düren, Germany). Solvent: 40 mM sodium phosphate buffer pH 3.7/MeOH 60:40, flow: 0.2 mL/min, pressure: 48 bar. **4** and **10** were detected at 249 nm, and **7** at 210 nm. Retention times: (+)-**4**: 100.2 min, (−)-**4**: 117.1 min, $\alpha = 1.17$; **7** (first peak): 38.7 min, **7** (second peak) 46.6 min, $\alpha = 1.20$; (*rac*)-**5**: 25.4 min; (*rac*)-**6**: 23.1 min; (+)-**10**: 72.9 min, (−)-**10**: 78.9 min, $\alpha = 1.08$. The conversion of the hydrolysis of (*rac*)-**4** to **10** was calculated according to the equation $c = ee_s/(ee_s + ee_p)$,[16] where c means the conversion, ee_s the enantiomeric excess of (−)-**4**, and ee_p the enantiomeric excess of (+)-**10**.

All chemicals were reagent grade and were used without further purification. Enzymes are commercially available; for details, see the Supporting Information.

Assays for Enzymatic Activity. Standard assays for enzyme activities were carried out as described by Horgan et al. for PLE[21] and as described by the supplier for lipases.[20] One unit (U) is defined as the amount of enzyme required to catalyze the hydrolysis of 1 μmol substrate per minute.

2-Azabicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic Acid 2-*tert*-Butyl Ester 6-Methyl Ester ((*rac*)-4) and 5-Azatricyclo-[4.1.0.0^{2,4}]heptane-3,5,7-tricarboxylic Acid 5-*tert*-Butyl Ester 3,7-Dimethyl Ester ((*rac*)-5). To a solution of *N*-*tert*-butoxycarbonylpyrrole **3a** (22.0 g, 131.7 mmol) in CH_2Cl_2 (100 mL) were added copper(II) triflate (100 mg, 0.28 mmol, 0.2 mol %) and phenylhydrazine (100 μL of a solution (1 wt %) in CH_2Cl_2) at room temperature under a nitrogen atmosphere. After being stirred for 20 min, a solution of methyl diazoacetate (16.50 g, 165 mmol) in CH_2Cl_2 (200 mL) was added slowly via a dropping funnel (ca. 1 drop/2 s). After the addition was complete (6 h), the mixture was further stirred for 12 h at room temperature. To remove the catalyst the mixture was filtered through silica gel, and the silica gel was washed with CH_2Cl_2. After evaporation of the solvent, the residue was purified by flash chromatography (hexanes/ethyl acetate 20:1 (ca. 1 L) followed by 10:1 to yield I: **3a** (8.80 g, 36%, $R_f = 0.65$ in hexanes/ethyl acetate 10:1). II: (*rac*)-**4** (14.2 g, 45%, $R_f = 0.26$) containing dimethyl fumarate (ca. 6%) as impurity, which was removed by sublimation (40 °C, 0.01 Torr, 6 h) to yield 12.30 g (39%) of pure (*rac*)-**4** as a colorless oil which solidifies after prolonged storage at −20 °C (mp = 48 °C): ^{1}H NMR (250 MHz, $CDCl_3$, signal doubling because of rotamers) δ 0.92 (br s, 1H), 1.46 (s, 9H), 2.77 (br s, 1H), 3.62 and 3.65 (s, 3H), 4.26−4.40 (m, 1H), 5.28−5.36 (m, 1H), 6.40−6.55 (br s, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$, signal doubling because of rotamers) δ 22.62 and 22.76, 28.11, 30.91 and 32.16, 44.06 and 44.19, 51.72, 81.61, 109.78, 129.55 and 129.74, 150.84 and 151.14, 173.23 and 173.50; MS (DCI(NH_3)) 257 (100) [$M^+ + NH_3 + H$]. Anal. Calcd for $C_{12}H_{17}NO_4$: C, 60.24; H, 7.16. Found: C, 60.46; H, 7.17. III: mixture of (*rac*)-**5** and dimethyl maleate (3.72 g, $R_f = 0.09$). This mixture was treated with cyclopentadiene (2.5 mL) in CH_2Cl_2 (10 mL) for 12 h and purified by flash chromatography (hexanes/ethyl acetate 10:1) to yield 1.37 g (3%) pure (*rac*)-**5** as a colorless oil, which slowly crystallized from a mixture CH_2Cl_2/cyclohexane at −20 °C (mp = 90 °C): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.71−1.73 (m, 2H), 2.28−2.32 (m, 2H), 3.31−3.33 (m, 1H), 3.45−3.48 (m, 1H), 3.58 (s, 3H), 3.62 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 26.62, 27.40, 27.58, 28.12, 28.85, 42.03, 51.77, 80.92, 154.14, 170.50, 170.75; HRMS (DCI(NH_3)) calcd for $[C_{15}H_{21}NO_6]^+$ 311.1373. Found 311.1369.

For large-scale preparations (*rac*)-**4** can also be obtained in pure form by distillation (10^{-4} Torr, 70−80 °C) after removal of the catalyst by filtration on silica gel.

(1*R,2*R**,3*R**)-*N*-*tert*-Butoxycarbonyl-*N*-formyl-3-aminocyclopropane-1,2-dicarboxylic Acid Dimethyl Ester ((*rac*)-6).** A solution of (*rac*)-**4** (478 mg, 2.0 mmol), 100 mg of $NaHCO_3$, and 1.0 mL of MeOH in CH_2Cl_2 (35 mL) was treated with ozone at −78 °C until the solution maintained a blue color. After removal of the excess ozone by passing oxygen through the solution, Ac_2O (0.5 mL) and NEt_3 (1.0 mL) were added slowly. The solution was allowed to warm to room temperature and stirred for another 6 h. The reaction solution was extracted with water and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by flash chromatography (hexanes/ethyl acetate 5:1, $R_f = 0.23$) to yield 469 mg (78%) (*rac*)-**6** as a colorless oil, which crystallized from a mixture CH_2Cl_2/cyclohexane at −20 °C as a white solid (mp = 64 °C): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.51 (s, 9H), 2.61−2.64 (m, 2H), 3.18 (dd, $J = 6.7$, 6.0 Hz, 1H), 3.68 (s, 3H), 3.75 (s, 3H), 9.11 (s, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 27.82, 28.08, 29.01, 35.45, 52.35, 52.54, 84.83, 151.96, 163.27, 168.53, 170.19; MS (EI(70 eV)) 301 (1) [M^+], 57 (100) [$C_4H_9^+$]. Anal. Calcd for $C_{13}H_{19}NO_7$: C, 51.82; H, 6.36; N, 4.65. Found: C, 52.03; H, 6.30, N, 4.72.

(1*R,2*R**,3*R**)-*N*-*tert*-Butoxycarbonyl-3-aminocyclopropane-1,2-dicarboxylic Acid Dimethyl Ester ((*rac*)-7).** To a solution of (*rac*)-**6** (301 mg, 1.0 mmol) in MeOH (5 mL) were added $KHCO_3$ (120 mg, 1.2 mmol) and water (3 mL). The mixture was stirred for 3 h at room temperature. After addition of water (5 mL), the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, and the residue was recrystallized from CH_2Cl_2/Et_2O to yield 231 mg (85%) of (*rac*)-**7** as a colorless solid (mp = 86 °C): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.39 (s, 9H), 2.22 (dd, $J = 5.0$, 5.0 Hz, 1H), 2.43 (dd, $J = 8.3$, 5.0 Hz, 1H), 3.66 (s, 3H), 3.70 (s, 3H), 3.72 (br s, 1H), 5.53 (br s, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 26.11, 28.15, 28.58, 37.33, 52.21, 52.33, 80.19, 155.29, 170.07; MS (EI(70 eV)) 273 (1) [M^+], 57 (100) [$C_4H_9^+$]. Anal. Calcd for $C_{12}H_{19}NO_6$: C, 52.74; H, 7.01; N, 5.13. Found: C, 52.29; H, 7.20; N, 5.11.

General Procedures for Enzyme Screening for the Hydrolysis of (*rac*)-4 and (*rac*)-7. (a) Esterases (Aqueous Suspension). Substrate suspensions: Approximately 8 mg (29 μmol) of finely powdered (*rac*)-**7** was suspended in 2.0 mL of potassium phosphate buffer (0.1 M, pH 7.4) by ultrasonication. (*rac*)-**4** could neither be powdered nor suspended in the aqueous solvent. To prepare a homogeneous and stable suspension approximately 7 mg of (*rac*)-**4** (29 μmol) were dissolved in 20 μL of acetone, and 2.0 mL of potassium phosphate buffer (0.1 M, pH 7.4) was added. Finally, the mixture was vigorously shaken and ultrasonicated.

Bioconversions: About 10 U of esterase (see Tables 1 and 2) was added to each of the substrate suspensions, and the resulting reaction mixtures were stirred at ambient temperature. During the investigated period of time samples of 100 μL were taken and acidified with 12 μL of 1.0 M phosphoric acid to a pH of 2.5−3.5 and immediately extracted with 200 μL of ethyl acetate. The conversion of the reaction was monitored by analysis of the organic phase after centrifugation with qualitative TLC.

(b) Screening for Lipases (Biphasic System). 10 mM solutions of (*rac*)-**4** and (*rac*)-**7**, respectively, in toluene were used. 800 μL of substrate solution, 1200 μL potassium phosphate buffer (0.1 M, pH 7.4) and 25 mg lipase (see Tables 1 and 2) were stirred at ambient temperature. During the investigated period of time three samples of each 250 μL were taken out of the reaction mixture. After centrifugation the toluene phase was separated, 75 μL of the aqueous phase was acidified with 9 μL of 1.0 M phosphoric acid to pH 2.5−3.5 and immediately extracted with 150 μL of ethyl acetate. The conversion of the reaction was monitored by analysis of the organic phase with qualitative TLC.

General Procedure for the Determination of the Specific Activity and Enantiomeric Ratio (*E* Value) of Active Enzymes for the Hydrolysis of (*rac*)-4 and (*rac*)-7. (a) Reactions Carried out in Aqueous Suspension (Esterases). Substrate suspensions: 42.0 mg (154 μmol) of finely powdered (*rac*)-**7** was suspended with stirring in 20.0 mL of potassium phosphate buffer (0.1 M, pH 7.4). 45.0 mg (188 μmol) of (*rac*)-**4** was dissolved in 125 μL of acetone, and

(20) Boehringer Mannheim GmbH. *Instructions to Chirazyme lipases & esterases screening set* **1996**.

(21) Horgan, D. J.; Stoops, J. K.; Webb, E. C.; Zerner, B. *Biochemistry* **1969**, *8*, 2000−2006.

(22) Merck, E. *Anfärbereagenzien für Dünnschicht und Papier-Chromatographie*; E. Merck: Darmstadt, 1970; p 29.

500 μL of a solution of Triton X-100 in water (10 g/L) was added to stabilize the emulsion. 2.5 mL of potassium phosphate buffer (1.0 M, pH 7.4) and water were added to a final volume of 25 mL (final buffer concentration 0.1 M). This mixture was vigorously shaken and then ultrasonicated for 15 min. During ultrasonication, (*rac*)-**4** precipitated to form a white colored, stable suspension.

Bioconversions: 20 mL of the substrate suspensions was kept at 25 °C, and the reactions were started by addition of the investigated enzyme. For the hydrolysis of (*rac*)-**4** (i) 150 U of PLE A, (ii) 70000 U of L-1, (iii) 1440 U of L-2, and (iv) 80000 U L-6 were used. 150 U of PLE A were used for the hydrolysis of (*rac*)-**7**.

The pH of the reaction mixtures was maintained at 7.5 by pH-stat-controlled addition of 0.1 M aqueous NaOH. The conversion was calculated from the amount of base consumed during the reaction.

The enantioselectivity of the kinetic resolution of (*rac*)-**7**, catalyzed by PLE and L-2, was investigated after extracting 500 μL samples with the same amount of cyclohexane. The organic layer was analyzed by chiral GC.

The product mixture **8** and **9** of the PLE-catalyzed hydrolysis of (*rac*)-**7** (100% conversion) was isolated as follows: After adjusting the pH to 8.0 by addition of 0.1 M aqueous NaOH the reaction mixture was extracted with Et_2O. The aqueous solution was acidified to pH 2.5 by addition of 1.0 M phosphoric acid. After saturation with NaCl, the mixture was extracted with Et_2O (3x), and the combined organic layers were extracted with brine and water. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to yield 36.3 mg (91%) of a mixture of **8** and **9** (60:40 by NMR and HPLC) as a slightly yellow oil which crystallized during storage for several weeks at room temperature.

The specific activity of PLE A for the conversion of (*rac*)-**7** was determined to be 0.21 U/mg.

(b) Reactions Carried out in a Biphasic System (Lipases). A 10 mM solution of (*rac*)-**4** in toluene and a solution of 62.5 mg lipase (L-1, L-2, L-6) in 3000 μL potassium phosphate buffer (0.1 M, pH 7.4) were prepared. Eight aliquot starting mixtures, each consisting of 300 μL of lipase solution and 200 μL of substrate solution ((*rac*)-**4** in toluene), were stirred at ambient temperature and worked up after different reaction times as follows: The reaction mixture was diluted with 1000 μL of toluene and acidified to pH 2.5−3.5 by addition of 30 μL of 1.0 M phosphoric acid. After vigorous shaking and subsequent centrifugation the toluene layer was analyzed by quantitative TLC for the determination of the conversion and by GC for the determination of the enantiomeric excess of **4**.

Optimization of the Reaction Conditions for the L-2-Catalyzed Hydrolysis of (*rac*)-4. General procedure: (for detailed amounts and solvents see the Supporting Information, Table 4): 5 mg (21 μmol) of (*rac*)-**4** was dissolved in the respective solvent. An aqueous solution of Triton X-100 in water (10 g/L), potassium phosphate buffer (pH 7.4) and a freshly prepared solution of L-2 in 0.1 M phosphate buffer, pH 7.4 (60 mg/mL) were added. After homogenization by vigorous shaking, the reaction mixture was stirred at ambient temperature. During the investigated period of time samples of 100 μL each were taken out of the freshly homogenized reaction mixture. After addition of 500 μL of Et_2O and 10 μL of 1.0 M phosphoric acid, the resulting mixture was shaken vigorously and then centrifugated. 100 μL of the resulting Et_2O layer was transferred to a HPLC-vial and the solvent was removed by passing over a gentle stream of nitrogen. The resulting semi-dry sample was dried in vacuo (14 mbar) and 1000 μL of HPLC solvent (see the general methods) were added subsequently. After shaking for 1 h at room temperature on a thermostatic mixer the sample was analyzed by chiral HPLC for determination of the conversion and the enantiomeric excesses of **4** and **10**. For the optimization of the pH, phosphate buffers of pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 were used instead of the phosphate buffer, pH 7.4.

Preparative Bioconversion of (*rac*)-4 Using L-2. Buffer. 50 mL of 1.0 M potassium phosphate buffer (pH 7.4) and 11.1 mL of an aqueous solution of Triton X-100 (10 g/L) were mixed together, and water was added to a final volume of 500 mL (final buffer concentration 0.1 M).

Enzyme Solution. 120 mg of L-2 was thoroughly suspended in 2 mL of 0.1 M potassium phosphate buffer (pH 7.4) and left overnight at 4 °C to form a clear solution.

2-Azabicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic Acid 2-*tert*-Butyl Ester 6-Methyl Ester ((−)-4). In a 1 L round-bottom flask 360 mL of buffer and a solution of 1.0 g (4.18 mmol) of (*rac*)-**4** in 40 mL of toluene were mixed together and vigorously stirred. The reaction was started by addition of the enzyme solution prepared above, and the resulting mixture was stirred at ambient temperature with protection from sunlight. Samples of 200 μL were diluted with 200 μL of toluene and the organic phase was analyzed by Chiral-GC. After 196 h (+)-**4** was completely hydrolyzed. The reaction mixture was acidified to pH 3.0 by addition of 1.0 M phosphoric acid followed by addition of NaCl (125 g). The mixture was immediately extracted with Et_2O (4×). The combined organic layers were extracted with brine and several times with 1 M $KHCO_3$ solution until TLC analysis indicated the absence of **10** in the organic layer. The organic layer was washed with water, dried over $MgSO_4$, and purified by filtration using a short column of silica gel (20 g, Et_2O as eluent) to yield 335 mg (33%) of (−)-**4** as an amber-colored oil that crystallized upon storage at −20 °C, ee = 99.6% (HPLC, GC, for conditions and retention times see general experimental part): $[\alpha]^{21}_D = -254.2$ (*c* 1.007, CH_2Cl_2). For further analytical data cf. preparation of (*rac*)-**4**.

The combined aqueous layers were extracted with cyclohexane, adjusted carefully to pH 3.0 by addition of aqueous HCl (2.5 M) and 1.0 M phosphoric acid, and the resulting suspension was extracted with Et_2O. The combined organic layers were dried and concentrated to yield 462 mg (49%) (+)-**10** as a colorless solid, ee = 43.3% (HPLC, for conditions and retention times see the general methods), $[\alpha]^{25}_D = +107.3$ (*c* 0.122, $CHCl_3$).

Preparative Bioconversion of (*rac*)-4 by L-2. Substrate solution: 1.067 g of (*rac*)-**4** was dissolved in 43 mL of cyclohexane.

Enzyme solution: 60 mg of L-2 was thoroughly supended in 1000 μL of 0.1 M potassium phosphate buffer, pH 7.0, and left for 1 h at 4 °C to form a clear solution.

2-Azabicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic Acid 2-*tert*-Butyl Ester ((+)-10). The substrate solution was added to 384 mL of 0.1 M potassium phosphate buffer, pH 5.0 and the biphasic mixture was stirred vigorously in a 1 L round-bottom flask. The reaction was started by addition of the enzyme solution and the reaction vessel was protected from sunlight and stirred at ambient temperature. Samples of 100 μL were diluted with 200 μL of toluene, 15 μL of 1 M $KHCO_3$ solution were added, and the organic phase was analyzed by GC (which is faster than HPLC). After 19.5 h **4** displayed an enantiomeric excess of ee_S = 48.4% corresponding to an calculated ee_P of 90.3% (E = 31.8). The reaction was stopped by addition of 10 mL of 1.0 M phosphoric acid and 300 mL of Et_2O. The layers were separated and the organic phase was washed with saturated NaCl solution and extracted with Et_2O. The combined organic layers were extracted several times with 50 mL portions of 1 M $KHCO_3$ solution until TLC indicated absence of acid **10** in the organic phase. The ether phase was finally washed with water, dried over $MgSO_4$ and concentrated to yield 711 mg (67%) (−)-**4** as an amber-colored oil: ee = 48.5% (GC, HPLC); $[\alpha]^{25}_D = -114.5$ (*c* 0.220, $CHCl_3$).

The combined $KHCO_3$ layers were washed with Et_2O, acidified to pH 3.5 by addition of aqueous HCl (2.0 M) and to pH 3.0 by addition of 1.0 M phosphoric acid. The resulting aqueous solution of acid (+)-**10** was subsequently extracted with Et_2O. The combined ether layers were dried over $MgSO_4$ and concentrated to yield 303 mg (30%) (+)-**10** as a colorless solid. 91.0% ee (GC, determined as methyl ester (+)-**4** after derivatization with trimethylsulfonium hydroxide). Upon recrystallization of 100 mg of (+)-**10** from hexane/ethyl acetate 18 mg (+)-**10** crystallized with 60% ee**,** leaving 82 mg of enantiomerically pure (+)-**10** (>96% ee) in the mother liquor: $[\alpha]^{25}_D = +255.1$ (*c* 0.127, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$, signal doubling because of rotamers) δ 0.92 (br s, 1H), 1.48

and 1.51 (s, 9H), 2.87 (br s, 1H), 4.31−4.50 (m, 1H), 5.36−5.40 (m, 1H), 6.45−6.61 (m, 1H), 11.87 (br s, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$, signal doubling because of rotamers) δ 22.95, 28.15, 31.87 and 33.10, 44.77 and 45.00, 82.02, 109.83, 129.73 and 129.98, 150.96 and 151.15, 179.18 and 179.75; HRMS (DCI(NH_3)) calcd for $[C_{11}H_{15}NO_4]^+$ 225.1006, found 225.1001.

(1*R*,2*R*,3*S*)-*N*-*tert*-Butoxycarbonyl-3-aminocyclopropane-1,2-dicarboxylic Acid 2-Monomethyl Ester (8). To a solution of (+)-**10** (461 mg, 2.05 mmol) in MeOH (35 mL) was added $NaHCO_3$ (344 mg, 4.1 mmol), and the mixture was stirred for 25 min at room temperature (gas evolution) before treatment with ozone at −78 °C until the solution maintained a blue color. After removal of the excess ozone by passing oxygen through the solution, the solution was allowed to warm to room temperature overnight. The solvent was evaporated, and to the residue was added benzene (2 × 20 mL) before evaporating again. The residue was dissolved in CH_2Cl_2 (35 mL), cooled to −78 °C, and Ac_2O (1.0 mL) and NEt_3 (2.0 mL) were added. The solution was allowed to warm to room temperature overnight again, diluted with $CHCl_3$ and extracted with 1 M $KHSO_4$ solution. The organic layer was dried over Na_2SO_4 and concentrated. The residue was dissolved in CH_3CN (10 mL) and DEAEA (0.83 mL, 5.8 mmol) was added. The solution stirred for 24 h at room temperature. The solvent was evaporated and the residue was dissolved in 20 mL ethyl acetate. After acidification with 20 mL of 1 M $KHSO_4$ solution, the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated to yield 352 mg (66%) **8** as a colorless solid (> 96% ee according to chiral HPLC analysis; mp = 147 °C): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.42 (s, 9H), 2.19 (br s, 1H), 2.46−2.52 (m, 1H), 3.74 (s, 3H), 3.83 (br s, 1H), 5.78 (br s, 1H), 11.34 (br s, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 25.80, 28.17, 28.95, 37.38, 52.53, 80.94, 156.10, 170.60, 173.61; MS (DCI(NH_3)) 277 (100) [$M^+ + NH_3 + H$]. Anal. Calcd for $C_{11}H_{17}NO_6$: C, 50.96; H, 6.61. Found: C, 51.06; H, 6.72. $[\alpha]^{21}_D = +48.7$ (*c* 0.980, CH_2Cl_2).

(1*S*,2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-3-aminocyclopropane-1,2-dicarboxylic Acid 2-Monomethyl Ester ((*ent*)-9). To a solution of **12** (1.13 g, 3.93 mmol) in CH_3CN (30 mL) was added DEAEA (1.15 mL, 8.06 mmol). The reaction was stirred for 24 h at room temperature. The solvent was evaporated, and the residue was dissolved in ethyl acetate (15 mL). The solution was acidified with 15 mL of 1 M $KHSO_4$ and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated to yield 889 mg (87%) (*ent*)-**9** as a colorless solid (mp = 139 °C): ^{1}H NMR (250 MHz, $CDCl_3$, signal doubling because of rotamers) δ 1.46 (s, 9H), 2.33 (dd, *J* = 4.9, 4.9 Hz, 1H), 2.41−2.46 (m, 1H), 3.42 and 3.88 (br s, 1H), 3.71 (s, 3H), 5.54 and 6.81 (br s, 1H), 11.61 (br s, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 26.78, 28.18, 29.44, 37.50, 52.34, 82.37, 158.30, 170.46, 172.06; MS (DCI(NH_3)) 536 (22) [$2M^+ + NH_3 + H$], 277 (100) [$M^+ + NH_3 + H$]. Anal. Calcd for $C_{11}H_{17}NO_6$: C, 50.96; H, 6.61. Found: C, 51.17; H, 6.56. $[\alpha]^{23}_D = -22.5$ (*c* 1.021, CH_2Cl_2).

(1*S*,2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-*N*-formyl-2-formyl-3-aminocyclopropanecarboxylic Acid Methyl Ester (11). A solution of (−)-**4** (800 mg, 3.34 mmol) in CH_2Cl_2 (50 mL) was treated with ozone at −78 °C until the solution maintained a blue color. After removal of the excess ozone by passing oxygen through the solution, dimethyl sulfide (1.04 g, 16.7 mmol) was added and the solution was stirred for 12 h at room temperature. *Caution: It is important that the time of reduction is sufficiently long, otherwise the reduction is incomplete.* After evaporation of the solvent the residue was purified by flash chromatography (hexanes/ethyl acetate 5:1, R_f = 0.19) to yield 827 mg of a colorless oil, which was crystallized from Et_2O (1.1 mL)/cyclohexane (2.9 mL) to afford 717 mg (79%) **11** as a white solid (mp = 59 °C): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.52 (s, 9H), 2.75 (dd, *J* = 6.0, 4.8 Hz, 1H), 2.96 (ddd, *J* = 8.2, 6.0, 2.3 Hz, 1H), 3.20 (dd, *J* = 8.2, 4.8 Hz, 1H), 3.75 (s, 3H), 9.07 (s, 1H), 9.54 (d, *J* = 2.3 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 27.68, 27.77, 34.86, 36.56, 52.50, 85.29, 151.83, 163.31, 169.91, 193.02; MS (DCI(NH_3)) 289 (100) [$M^+ + NH_3 + H$]. Anal. Calcd for $C_{12}H_{17}NO_6$: C, 53.13; H, 6.32; N, 5.16. Found: C, 53.02; H, 6.29; N, 5.02; $[\alpha]^{21}_D = -48.7$ (*c* 1.004, CH_2Cl_2).

(1*S*,2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-*N*-formyl-3-aminocyclopropane-1,2-dicarboxylic Acid 2-Monomethyl Ester (12). A solution of **11** (2.25 g, 8.23 mmol) in CH_3CN (25 mL) was cooled to 0 °C, and under rigorous stirring a solution of 666 mg KH_2PO_4 in 6.7 mL of water and 0.84 mL of H_2O_2 (30%) were added. After addition of a solution of 1.67 g of $NaClO_2$ (technical, 80%) in 16.7 mL water, the reaction solution stirred until ending of gas evolution (ca. 2 h). Then, 830 mg of Na_2SO_3 was added to destroy excess $NaClO_2$ and the solution stirred for 1 h. After addition of 20 mL of 1 M $KHSO_4$, the solution was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4. Evaporation of the solvent afforded 2.28 g (7.92 mmol, 96%) **12** as a colorless oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.51 (s, 9H), 2.59 (dd, *J* = 7.7, 5.9 Hz, 1H), 2.65 (dd, *J* = 5.9, 5.1 Hz, 1H), 3.22 (dd, *J* = 7.7, 5.1 Hz, 1H), 3.77 (s, 3H), 9.12 (s, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 27.74, 27.82, 29.52, 35.83, 52.68, 85.19, 151.87, 163.33, 169.89, 173.47; HRMS (DCI(NH_3)) calcd for $[C_{12}H_{18}NO_7]^+$ 288.1076, found 288.1083; $[\alpha]^{21}_D = -66.9$ (*c* 0.999, CH_2Cl_2).

(1*S*,2*S*,3*R*)-*N*-*tert*-Butoxycarbonyl-3-aminocyclopropane-1,2-dicarboxylic Acid 1-Benzyl Ester 2-Methyl Ester (13). To a solution of (*ent*)-**9** (1.91 g, 7.35 mmol) in DMF (37 mL) were added $NaHCO_3$ (1.24 g, 14.7 mmol) and benzyl bromide (1.38 g, 8.09 mmol). After being stirred for 48 h at room temperature, the solution was diluted with 100 mL of ethyl acetate and 100 mL of water. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were extracted with water and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography (hexanes/ethyl acetate 5:1, R_f = 0.38) to afford 2.46 g (96%) **13** as a colorless solid (mp = 93 °C): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.43 (s, 9H), 2.28 (dd, *J* = 5.0, 5.0 Hz, 1H), 2.51 (dd, *J* = 8.3, 5.0 Hz, 1H), 3.68 (s, 3H), 3.85 (br s, 1H), 5.11 (d, *J* = 12.2 Hz, 1H), 5.20 (d, *J* = 12.2 Hz, 1H), 5.55 (br s, 1H), 7.36 (s, 5H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 26.24, 28.17, 28.66, 37.48, 52.24, 67.22, 80.21, 128.26, 128.44, 128.57, 135.03, 155.27, 169.70, 170.01; MS (DCI(NH_3)) 367 (100) [$M^+ + NH_3 + H$]. Anal. Calcd for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64. Found: C, 61.93; H, 6.67. $[\alpha]^{21}_D = -8.9$ (*c* 1.000, CH_2Cl_2).

(1*S*,2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-2-[1′(*S*)-(4″-chlorophenyl)ethylcarbamoyl]-3-aminocyclopropanecarboxylic Acid Methyl Ester (14). To a solution of (*ent*)-**9** (100 mg, 0.39 mmol) in THF (10 mL) were added pentafluorophenole (142 mg, 0.77 mmol), (*S*)-*p*-chlorophenylethylamine (66 μL, 0.43 mmol), and EDC (148 mg, 0.77 mmol). The mixture was stirred for 20 h at room temperature. The reaction mixture was diluted with ethyl acetate and then washed with saturated $NaHCO_3$, 1 M $KHSO_4$, and saturated $NaHCO_3$ and dried over Na_2SO_4. The solvent was evaporated, and the resulting residue was purified by flash chromatography (hexanes/ethyl acetate 3:1, R_f = 0.14) to provide **14** (147 mg, 95%) as a colorless solid (mp = 149 °C): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.47 (dd, *J* = 6.9, 3H), 2.22−2.32 (m, 2H), 3.68 (s, 3H), 3.73 (br s, 1H), 5.02−5.11 (m, 1H), 5.89−5.92 (m, 1H), 6.50−6.53 (m, 1H), 7.21−7.32 (m, 4H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 21.74, 27.55, 27.68, 28.28, 37.72, 48.80, 52.23, 80.09, 127.44, 128.88, 133.27, 141.30, 155.75, 167.55, 171.17; MS (DCI(NH_3)) 414 (38) [$M^+ + NH_3 + H$], 397 (100) [$M^+ + H$], 793 (39) [$2M^+ + H$]. Anal. Calcd for $C_{19}H_{25}N_2O_5Cl$: C, 57.51; H, 6.44; N, 7.11. Found: C, 57.50; H, 6.35; N, 7.06. $[\alpha]^{21}_D = -112.4$ (*c* 1.020, CH_2Cl_2).

(1*R*,2*R*,3*R*)-*N*-*tert*-Butoxycarbonyl-3-aminocyclopropane-1,2-dicarboxylic Acid 1-Benzyl Ester 2-Methyl Ester (16). To a solution of **8** (86 mg, 0.332 mmol) in DMF (2 mL) were added $NaHCO_3$ (31 mg, 0.365 mmol) and benzyl bromide (0.58 g, 0.332 mmol). After being stirred for 48 h at room temperature, the solution was diluted with ethyl acetate and water. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were extracted with water and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by flash

chromatography (hexanes/ethyl acetate 5:1, $R_f = 0.38$) to afford 85 mg (74%) **16** as a colorless solid (>98% ee according to chiral HPLC analysis mp = 80 °C): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.43 (s, 9H), 2.28−2.32 (m, 1H), 2.49 (dd, $J = 8.4$, 5.2 Hz, 1H), 3.73 (s, 3H), 3.88 (br s, 1H), 5.08 (d, $J = 12.2$ Hz, 1H), 5.14 (d, $J = 12.2$ Hz, 1H), 5.53 (br s, 1H), 7.29−7.39 (m, 5H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 26.18, 28.18, 28.75, 37.48, 52.43, 67.13, 80.24, 128.37, 128.43, 128.57, 135.13, 155.30, 169.53; MS (DCI-(NH_3)) 367 (100) [$M^+ + NH_3 + H$]. Anal. Calcd for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64; N, 4.01. Found: C, 61.77; H, 6.68; N, 3.98. $[\alpha]^{21}_D = +10.2$ (*c* 1.000, CH_2Cl_2).

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Note Added in Proof: After the submission of this manuscript, a study on the enzymatic resolution of cyclopropane-*cis*-1,2-dicarboxylic methyl ester followed by Curtius degradation to arrive at *cis*-2-aminocyclopropanecarboxylic acid derivatives has been published: Martin-Vila, M.; Muray, E.; Aguada, G. P.; Alvarez-Larena, A.; Branchadell, V.; Minguillon, C.; Giralt, E.; Ortuno, R. M. *Tetrahedron: Asymmetry* **2000**, *11*, 3569.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of all new compounds together with X-ray crystallographic details for **14**. Detailed information on sources for enzymes and on optimization protocols for kinetic resolution of (*rac*)-**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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