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Selectivity of *Candida rugosa* lipase in simultaneous separation of skeletal isomers, desymmetrization, and kinetic racemate cleavage of 9-oxabicyclononanediols

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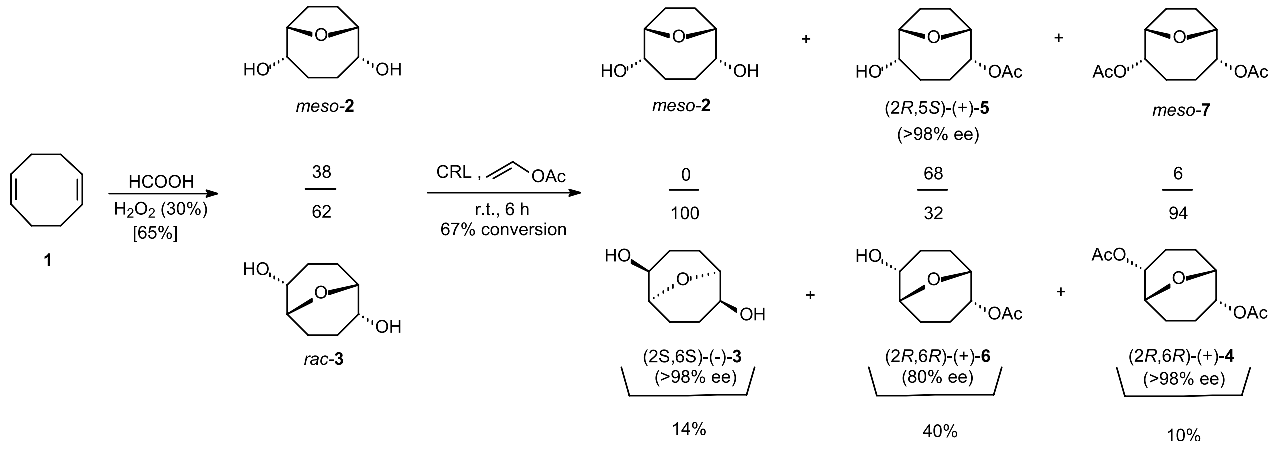
Abstract—The diols **2** and **3**, available in one step from cycloocta-1,5-diene, are selectively acetylated at the (*R*)-centers using *Candida rugosa* lipase to give the corresponding enantiopure compounds. In contrast, the (*S*,*S*)-enantiomer of **11** is transformed under identical conditions. The stereoselectivity of the lipase has been investigated by modeling of the transition state geometries at the active site of the enzyme. © 2003 Published by Elsevier Science Ltd.

Enantiopure diols, particularly C_2-symmetric ones, gain much interest as auxiliaries or sources for ligands of metal catalysts for asymmetric synthesis.[1] In several cases such compounds have been synthesized with high stereoselectivity by asymmetric alkylation of primary 1,*n*-alkanediols,[2] or by catalytic asymmetric[3] respective microbiological reduction[4] of the corresponding diketones. In such reactions, in addition to the desired enantiopure diols, frequently significant amounts of the *meso*-diastereomers have been formed. An alternative pathway is the differentiation of the stereoisomers by hydrolases. Applying this protocol the separation of several *meso*- and racemic secondary 1,*n*-diols was successful.[5] In numerous cases the selectivity of the applied enzymes was quite low or very different for the *meso*- and the racemic diols. In these cases the *meso*-compounds had to be separated prior to cleavage of the racemate,[6] or two subsequent enzyme-catalyzed separations have been necessary.[7]



Scheme 1.

Keywords: bicyclic diols; desymmetrization; enantiospecificity; lipase-catalyzed acetylation; molecular modeling; racemate cleavage.

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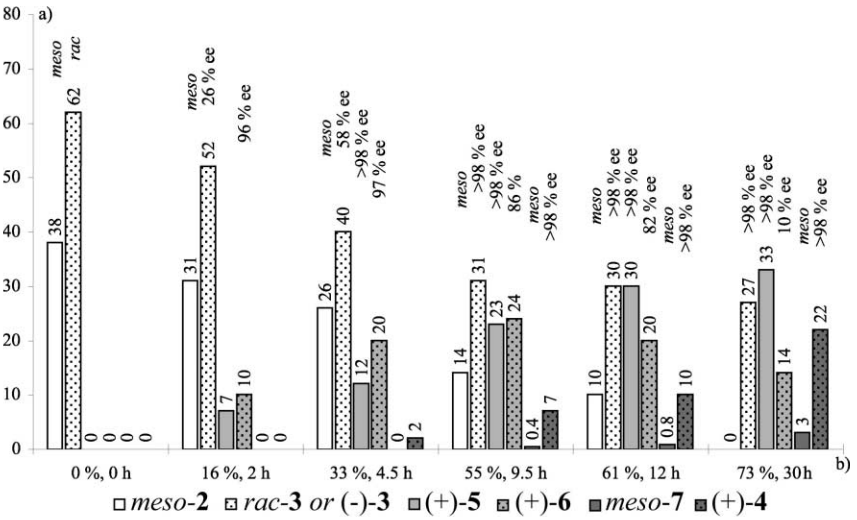


Figure 1. Time dependent conversion and development of the enantiomeric excesses of the products of CRL catalyzed acetylation of *meso*-**2** and *rac*-**3**.

In this letter, the first example of a lipase catalyzed simultaneous separation of skeletal isomeric diols, with kinetic resolution of the one and desymmetrization of the other constitutional isomer is reported. After screening of several lipases and esterases,[8] *Candida rugosa* lipase (CRL, Lipase Amano AY) has proved the most efficient enzyme for the separation of the isomeric diols **2** and **3**. The selectivity of this enzyme was investigated by means of molecular modeling of the transition state geometries using the X-ray structure of CRL, which has been determined some years ago.[9]

The acetylation[10] with vinyl acetate and CRL as the biocatalyst of a 38:62 mixture of *meso*-**2** and *rac*-**3**, which were prepared according to known procedures[11] by transannular *O*-heterocyclization of **1** with performic acid, reached 67% conversion after 6 hours. That means that the *meso*-compound **2** was transformed almost quantitatively, while the conversion of the racemate **3** stopped at 50% conversion. After separation of the enzyme, (1*S*,2*S*,5*S*,6*S*)-(−)-**3**[12,13] (14% yield, >98% ee), (1*R*,2*R*,5*R*,6*R*)-(+)-**4**[12,13] (10% yield, >98% ee, 6% of *meso*-**7** as an impurity) and 40% of a 68:32 mixture of (1*R*,2*R*,5*S*,6*S*)-(+)-**5**[12,13] (>98% ee) and (1*R*,2*R*,5*R*,6*R*)-(+)-**6**[12,13] (80% ee) were isolated (Scheme 1).

The conversion of **2** and **3** and the time dependent development of the enantiomeric excesses of the products are shown in Figure 1. For this experiment a slightly less active, but equally selective lipase preparation was used in order to guarantee the timely work up and analysis of the ratio of enantiomers.

As predicted by Kazlauskas' rule[14] in transformation of secondary alcohols or their derivatives with CRL, the enantiomer shown in Figure 2 was transferred preferred, which is the (*R*)-enantiomer in case that the larger substituent has also the higher priority according to the Cahn–Ingold–Prelog (CIP) rule.[15] This is true for the acetylation of **2** and **3**, and was observed earlier also in hydrolysis of *endo*,*endo*-2,6-diacetoxybicyclo[3.3.1]-nonane.[16]

Furthermore, it was most interesting to prove the enantioselectivity of CRL in the acetylation of the diastereomeric and also C_2-symmetric *exo*,*exo*-9-oxabicyclo[3.3.1]nonane-2,6-diol (**11**). This compound was prepared using a principally known[17] pathway. We started the five-step synthesis from *endo*,*endo*-2,6-diiodo-9-oxabicyclo[3.3.1]nonane (**9**)[18] using a reductive desulfuration of 2-oxa-6-thiatricyclo[3.3.1.1^{3,7}]decane-4,8-diol (**10**) as the final step (Scheme 2).

Using CRL and vinyl acetate, compound *rac*-**10** has been acetylated also (*R*)-selectively. The remaining diol (1*S*,3*S*,4*S*,5*S*,7*S*,8*S*)-(+)-**10**[13] showed >98% ee, while the monoacetate (1*R*,3*R*,4*R*,5*R*,7*R*,8*R*)-**12**[13] had 34% ee. For the diacetate (1*R*,3*R*,4*R*,5*R*,7*R*,8*R*)-(−)-**13**, 92% ee was detected.[13] Thus, this tricyclic dihetero adamantane derivative was also acetylated according to Kazlauskas' rule. The CH_2-CH-S- moiety is larger compared to the CH_2-CH-O- group and has also the higher priority in terms of the CIP rule (Scheme 2).

Contrary to all reactions mentioned above, the CRL catalyzed acetylation of *rac*-**11** was faster at the OH-groups attached to (*S*)-centers. The enantiomeric excess of the remaining diol, (1*S*,2*R*,5*S*,6*R*)-(+)-**11**,[12,13] was determined to 94% ee, while the monoacetate (1*R*,2*S*,5*R*,6*S*)-(−)-**14**[13] had only 30% ee and the diacetate (1*R*,2*S*,5*R*,6*S*)-(−)-**15**[13] showed 92% ee (Scheme 3).

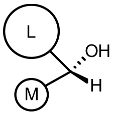
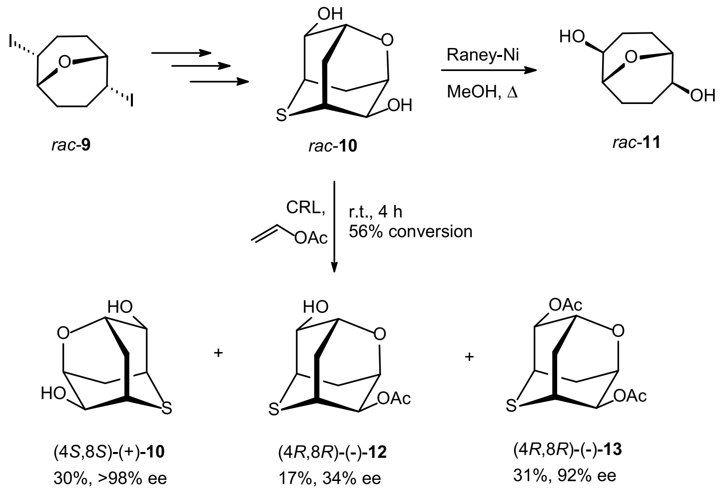
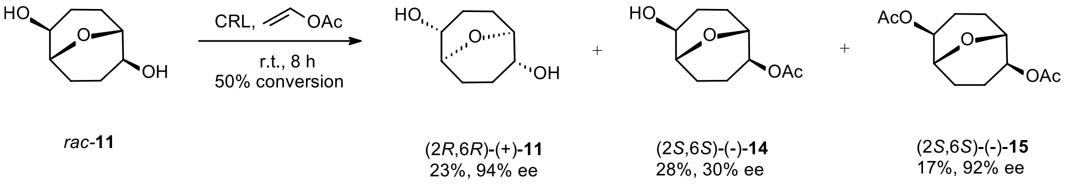


Figure 2. Enantiomer preferably transferred by lipases according to Kazlauskas' rule (adapted to the diols).[14b]



Scheme 2. Synthesis and lipase-catalyzed cleavage of racemic 2-oxa-6-thiaadamantane-4,8-diol (**10**) and synthesis of *exo*,*exo*-9-oxabicyclo[3.3.1]nonane-2,6-diol (**11**).



Scheme 3.

Closer examination of the most stable conformers calculated semiempirically (AM1)[19] of the five diols, makes their very similar geometry obvious (Fig. 3). Apparently, one enantiomer of these compounds can fit perfectly into the active site of the enzyme, even when the absolute configuration of the OH-bearing centers of diastereomeric compounds is different (cases of (1*R*,2*R*,5*R*,6*R*)-**3** and (1*R*,2*S*,5*R*,6*S*)-**11**, respectively).

The known X-ray crystal structures of CRL[9] with and without (1*R*)- and (1*S*)-menthylhexyl phosphonate as transition state analogues have been edited for modeling[20–22] and used for the construction of the respective structures of the acetylation of **2** and of the enantiomers of **3**.[23,24]

Using the energetically most stable geometries of each pair of enantiomers (or the enantiotopic groups of **2**) relative energies have been calculated. The energy difference for the model substrate menthol amounts 3.3 kcal/mol, while for the acetylation of the first OH group of *endo*,*endo*-**2** 2.6 kcal/mol and of *endo*,*endo*-**3** 0.9 kcal/mol were calculated in favor of the respective (*R*)-enantiomers (Fig. 4).

These force field calculations using a quite simple model of the active site of the enzyme (isolated protein, space-restricted optimization, no solvent), are therefore suitable for the determination of the absolute configuration of the preferably acetylated isomers of the model substrate menthol[9] as well as for the polycyclic diols **2** and **3**.

Experimental

A 38:62 mixture of compounds **2** and **3** (10.0 g, 63.3 mmol) in vinyl acetate (250 ml) was treated with CRL (2.5 g) and stirred at 20°C for 6 h (67% conversion). The lipase was filtered off using a short column with silica gel and vinyl acetate was removed in vacuo. The residue was dissolved in ethyl acetate (60 mL) and successively treated with *n*-pentane until no more of the non-reacted diol (–)-**3** precipitated. After separation of (–)-**3** the solvent was evaporated and the residue was

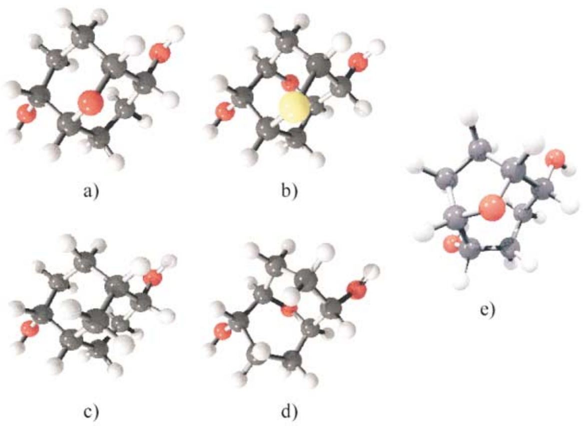


Figure 3. Most stable conformers of diol enantiomers transformed by *Candida rugosa* lipase. (a) (+)-**3**; (b) (–)-**10**); (c) (1*S*,2*R*,5*S*,6*R*)-(+)-bicyclo[3.3.1]nonane-2,6-diol; (d) (–)-**11**; (e) *meso*-**2**.

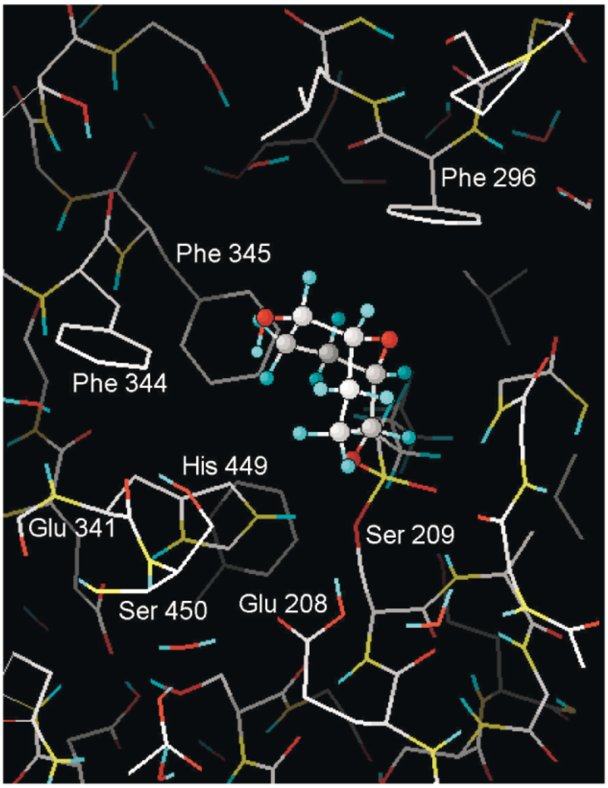


Figure 4. Arrangement of transition state analogous phosphate of (1*R*,2*R*,5*R*,6*R*)-(+)-**3** at the active site of *Candida rugosa* lipase.

chromatographed (silica gel, cyclohexane/ethyl acetate, 1:1) in order to separate compounds (+)-**5** and (+)-**6** from (+)-**4**. The diols *rac*-**10** and *rac*-**11** were treated analogously.

Acknowledgements

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References

1. (a) Kagan, H. B.; Dang, T. P. *J. Am. Chem. Soc.* **1972**, *94*, 6429–6433; (b) Whitesell, J. K. *Chem. Rev.* **1989**, *89*, 1581–1590; (c) Alexakis, A.; Mangeney, P. *Tetrahedron: Asymmetry* **1990**, *1*, 477–511; (d) Noyori, R. *Asymmetric Catalysis in Organic Synthesis*; Wiley & Sons: New York, 1994; (e) Nieman, J. A.; Keay, B. A. *Tetrahedron: Asymmetry* **1996**, *7*, 3521–3526.
2. Ahrens, H.; Paetow, M.; Hoppe, D. *Tetrahedron Lett.* **1992**, *33*, 5327–5330.
3. (a) Kitamura, M.; Ohkuma, T.; Inoue, S.; Sayo, N.; Kumobayashi, H.; Akutagaura, S.; Ohta, T.; Takaya, H.; Noyori, R. *J. Am. Chem. Soc.* **1988**, *110*, 629–631; (b) Aldous, D. J.; Dutton, W. M.; Steel, P. G. *Tetrahedron:Asymmetry* **2000**, *11*, 2455–2462.
4. (a) Lieser, J. K. *Synth. Commun.* **1983**, *13*, 765–767; (b) Fauve, A.; Veschambre, H. *Biocatalysis* **1990**, *3*, 95–109; (c) Otten, S.; Fröhlich, R.; Haufe, G. *Tetrahedron: Asymmetry* **1998**, *9*, 189–191 and references cited therein.
5. (a) Wallace, J. S.; Baldwin, B. W.; Morrow, C. J. *J. Org. Chem.* **1992**, *57*, 5231–5239; (b) Mattson, A.; Öhrner, N.; Hult, K.; Norin, T. *Tetrahedron: Asymmetry* **1993**, *4*, 925–930; (c) Mattson, A.; Orrenius, C.; Öhrner, N.; Unelius, C. R.; Hult, K.; Norin, T. *Acta Chim. Scand.* **1996**, *50*, 918–921; (d) Alexandre, F.-R.; Huet, F. *Tetrahedron: Asymmetry* **1998**, *9*, 2391–2410; (e) Matsumoto, K.; Sato, Y.; Shimjo, M.; Hatanaka, M. *Tetrahedron:Asymmetry* **2000**, *11*, 1965–1973; (f) Gung, B. W.; Dickson, H.; Shockley, S. *Tetrahedron Lett.* **2001**, *42*, 4761–4763.
6. (a) Guo, Z.-W.; Wu, S.-H.; Chen, C.-S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 4942–4945; (b) Caron, G.; Kazlauskas, R. J. *Tetrahedron: Asymmetry* **1994**, *5*, 657–664.
7. Kim, M.-J.; Lee, J. S.; Jeong, N.; Choi, Y. K. *J. Org. Chem.* **1993**, *58*, 6483–6485.
8. Hegemann, K. Ph.D. Thesis, Universität Münster, 1997.
9. (a) Grochulski, P.; Li, Y.; Schrag, J. D.; Bouthillier, F.; Smith, P.; Harrison, D.; Rubin, B.; Cygler, M. *J. Biol. Chem.* **1993**, *268*, 12843–12847; (b) Cygler, M.; Grochulski, P.; Kazlauskas, R. J.; Schrag, J. D.; Bouthilier, F.; Rubin, B.; Serregi, A. N.; Gupta, A. K. *J. Am. Chem. Soc.* **1994**, *116*, 3180–3186.
10. For an excellent review on enzyme-catalyzed syntheses in organic solvents, see: Carrea, G.; Riva, S. *Angew. Chem.* **2000**, *112*, 2312–2341; *Angew. Chem., Int. Ed.* **2000**, *39*, 2226–2254.
11. (a) Lafont, P.; Vivant, G. Fr. Pat. 1,336,187, 1963 [*Chem. Abstr.* **1963**, *60*, 2803]; (b) Frazer, A. H. US Pat. 3,347,826, 1967 [*Chem. Abstr.* **1968**, *68*, P2819]; (c) Duthaler, R. O.; Wicker, K.; Ackermann, P.; Ganter, C. *Helv. Chim. Acta* **1972**, *55*, 1809–1829; (d) Eaton, P. E.; Millikan, R. *Synthesis* **1990**, 483–484.
12. The determination of the absolute configuration by X-ray crystal structure analysis of these compounds will be reported in detail elsewhere (Hegemann, K.; Fröhlich, R.; Haufe, G., in preparation).
13. The spectroscopic data of the isolated optically active compounds agreed with those observed for the racemic ones **3**,[11c] **4**,[11c] **5**,[11c] **6**,[11c] **10**,[17b] **11**,[11c] **13**[17b] and **15**.[11c] The enantiomeric excesses, if not stated otherwise, have been detemined, by chiral GC using a chiral β-Dex™ 120 phase, 170°C, isothermic. (1*S*,2*S*,5*S*,6*S*)-(−)-**3**: >98% ee, $[\alpha]^{20}_{D}=-45.8$ (*c* 1, ethanol). (1*R*,2*R*,5*S*,6*S*)-(+)-**5**: >98% ee (^{1}H shift spectrum with 100 mol% of $Eu(hfc)_3$), mixture with (1*R*,2*R*,5*R*,6*R*)-(+)-**6**: 80% ee (^{1}H shift spectrum with 100 mol% of $Eu(hfc)_3$). $[\alpha]^{20}_{D}$ of the 68:32 mixture = +31.4 (*c* 1, ethyl acetate). (1*S*,2*S*,5*S*,6*S*)-(−)-**4**: Prepared from (1*S*,2*S*,5*S*,6*S*)-(−)-**3** by esterification with acetic anhydride in pyridine; >98% ee (GC, after hydrolysis), $[\alpha]^{20}_{D}=-53.7$ (*c* 1, ethyl acetate). (1*S*,3*S*,4*S*,5*S*,7*S*,8*S*)-(+)-**10**: >98% ee (GC, 190°C), $[\alpha]^{20}_{D}=+22.5$ (*c* 1, ethanol). (1*S*,2*R*,5*S*,6*R*)-(+)-**11**: 94% ee, $[\alpha]^{20}_{D}=+22.5$ (*c* 1, ethanol). (1*R*,3*R*,4*R*,5*R*,7*R*,8*R*)-(−)-**12**: mp 144°C (ethyl acetate), 34% ee (GC, 190°C, after hydrolysis), $[\alpha]^{20}_{D}=-17.6$ (*c* 1, ethyl acetate). (1*R*,3*R*,4*R*,5*R*,7*R*,8*R*)-(−)-**13**: 92% ee (GC, 190°C, after hydrolysis), $[\alpha]^{20}_{D}=-55.9$ (*c* 1, ethyl acetate). (1*R*,2*S*,5*R*,6*S*)-(−)-**14**: mp 70–71°C (ethyl acetate), 30% ee

(GC, after hydrolysis), $[\alpha]_D^{20}=-11.7$ (*c* 1, ethyl acetate). (1*R*,2*S*,5*R*,6*S*)-(−)-**15**: 92% ee (GC, after hydrolysis), $[\alpha]_D^{20}=-29.3$ (*c* 1, ethyl acetate).

14. (a) Oberhauser, T.; Faber, K.; Griengl, H. *Tetrahedron* **1985**, *45*, 1679–1682; (b) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656–2665; (c) Ahmed, S. N.; Kazlauskas, R. J.; Morinville, A. H.; Grochulski, P.; Schrag, J. D.; Cygler, M. *Biocatalysis* **1994**, *9*, 209–225.
15. (a) Cahn, R. S.; Ingold, C. K. Prelog, V. *Angew. Chem.* **1966**, *78*, 413–447; *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 385–419; (b) Prelog, V.; Helmchen, G. *Angew. Chem.* **1982**, *94*, 614–631; *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 567–584.
16. Naemura, K.; Matsumura, T.; Komatsu, M.; Hirose, Y.; Chikamatsu, H. *J. Chem. Soc., Chem. Commun.* **1988**, 239–241.
17. (a) Ganter, C.; Wicker, K. *Helv. Chim. Acta* **1968**, *51*, 1599–1603; (b) Stetter, H.; Meissner, H. J.; Last, W. D. *Chem. Ber.* **1968**, *101*, 2889–2893.
18. Haufe, G. *Tetrahedron Lett.* **1984**, *25*, 4365–4368.
19. (a) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stuart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909; (b) MOPAC7, AM1, QCPE Program No. 455.
20. The crystal structure for the force field calculations was prepared by decision of the ionization state of ionizable side chains and the orientation of ambivalent side chains.[21] Correspondingly the acidic hydrogen atoms were added. The partial charges of the heteroatom groups were obtained using the semiempirical MNDO method. All force field calculations were done with the AMBER implementation[22] of the modeling program MOBY in the 'united atom' approximation.[21]
21. Höweler, U. CHEOPS, 1999; the optimization was done using the MaxiMoby 5.2 program.
22. Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765–784.
23. For the conformational analysis for both enantiomers of **3** (or the enantiotopic OH-groups of **2**) the two torsions of the connection between the phosphate group (cf. Ref. 24) and the substituent (O-P-O-C-Sub and P-O-C-Sub-H-Sub) have been varied. Torsions have been run through in 10° steps for 360°. All conformations within 200 kcal/mol compared to the lowest minimum in the subsequent optimization have been taken into account. In this course the phosphate group, the substituent and the side chain of the amino acids of the active site (Glu-208, Ser-209 (bound to the phosphorus), Phe-296, Glu-341, Phe-344, Phe 345, His-449 and Ser-450) could adapt to each other.
24. Tuomi, W. V.; Kazlauskas, R. J. *J. Org. Chem.* **1999**, *64*, 2638–2647.