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Chemoenzymatic Methods for the Preparation of Optically Active Cyclic Polyazido Alcohols from Easily Available Achiral Starting Materials

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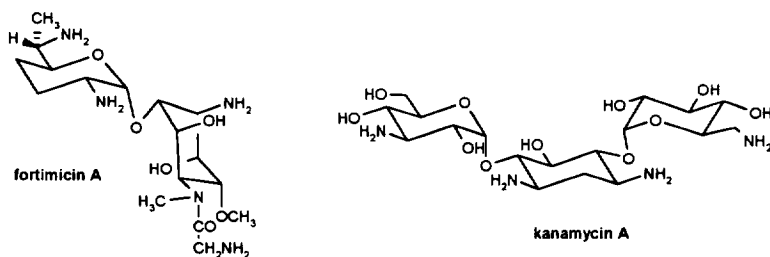
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Abstract: Optically active 2,5-diaziido-1,4-cyclohexanediol, 4,6-diaziido-1,3-cyclohexanediol and 2,5-diaziido-1,4-cyclohexanediol as precursors for polyfunctional cyclic amino alcohols were prepared by enzymatic hydrolysis of the respective butyrates with lipases from *Candida rugosa* (CRL), *Pseudomonas cepacia* (PCL), and *Geotrichum candidum* (GCL). The enantiomeric excesses obtained were very high. Copyright © 1996 Elsevier Science Ltd

Several pharmacologically active classes of compounds such as antibiotics contain the aminocyclitol moiety¹. The aminoglycoside antibiotics are the oldest known class of broad-spectrum antibacterials with activity against gram-positive and gram-negative organisms as well as mycobacteria. They are, however, not active against anaerobic bacteria or fungi².

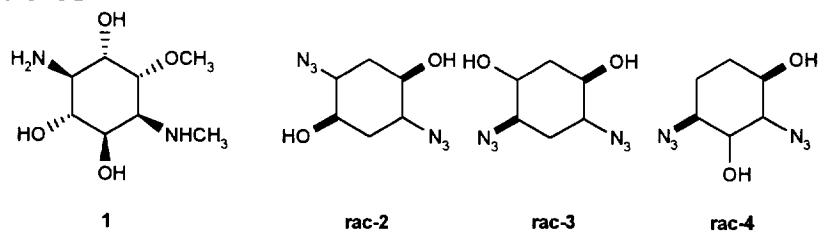
In fortamine **1**, the 1,4-diaminocyclitol moiety of fortimicin A (schemes 1 and 2), which was found in the fermentation broth of *Micromonospora olivoasterospora*³, all carbons of the cyclohexane ring are asymmetric, being substituted with oxygen or nitrogen functional groups⁴. The construction of such a polyfunctional cyclohexane derivative under stereo- and regiochemical control seemed quite interesting and challenging from a synthetic point of view. Some results indicate that all hydroxyl groups are not necessary for antimicrobial activity⁵. A problem common to most antibiotics is the emergence of resistant bacterial strains. Resistance to aminoglycosides is due primarily to the action of drug-modifying enzymes. Thus, new aminoglycosides have been prepared from established ones either by removing or alkylating functional groups that are targets of modifying enzymes⁶. In the literature the synthesis of chiral fortamines are already described⁷. In 1990 Kobayashi reported the first enantioselective synthesis of fortamine using pig liver esterase as chiral catalyst^{4,8}, while other groups used the chiral pool⁹. In 1992 Carless and collaborators described the enantiospecific synthesis by microbial oxidation of toluene¹⁰.

Scheme 1



In continuation of our earlier work¹¹, in this paper we describe the development of chiral cyclohexane synthons by a chemoenzymatic approach, and the preparation of some optically active stereoisomers of 2,4-diazido-1,5-cyclohexanediol **2**^{12,13} (precursor of 3,6-dideoxyfortamine), 4,6-diazido-1,3-cyclohexanediol **3**^{12,13} (derivative of 2-deoxystreptamine as part of several kanamycin antibiotics), and 2,4-diazido-1,3-cyclohexanediol **4**¹³ (derivative of 5,6-dideoxystreptamine).

Scheme 2

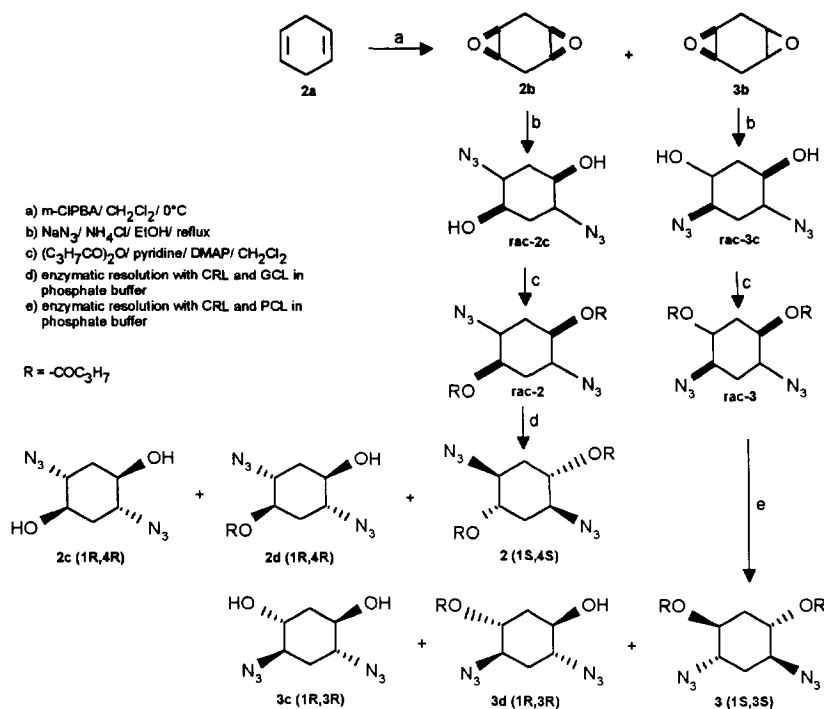


Besides the check on whether the envisaged synthetic strategy is a practical one for the synthesis of the compounds mentioned, our interest also is directed towards a deeper understanding of activities and selectivities of the enzymes used. Especially the possible regio- and stereoselection at the highly substituted substrates used triggered our interest.

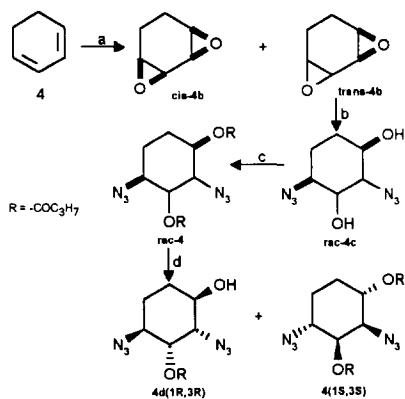
Methods

Bisepoxidation of 1,4-cyclohexadiene was carried out according to the literature^{14,16}. The preparation of the azidocyclitols **rac-2c** and **rac-3c** was carried out via ring-opening of the epoxides with sodium azide in ethanol. These ring-opening reactions are in accord with previous reports on the stereochemical course of the ring-opening reactions of these compounds with other nucleophiles and confirm the *trans* diaxial opening^{15,17} (scheme 3). The absolute configuration after the kinetic resolution step was estimated by comparison of the specific rotation values with similar known compounds¹⁸.

Scheme 3



Scheme 4



- a) $\text{CH}_3\text{COOOH}/\text{CH}_3\text{COONa}/\text{Na}_2\text{CO}_3/\text{CH}_2\text{Cl}_2$
 b) $\text{NaN}_3/\text{NH}_4\text{Cl}/\text{EtOH}/\text{water}$
 c) $(\text{C}_3\text{H}_7\text{CO})_2\text{O}/\text{pyridine}/\text{DMAP}/\text{CH}_2\text{Cl}_2$
 d) enzymatic resolution with CRL in phosphate buffer

Bisepoxidation of 1,3-cyclohexadiene with peroxyacetic acid in buffered solution was a convenient method for the synthesis of *trans*-bisepoxide **4b**, although the yield was very low (13%). The *cis*-bisepoxide was formed in a very small amount only. So for practical reasons only the *trans* isomer was used.

The azidocyclitol **rac-4c** was the only isomer built after ring-opening of *trans-4b* with sodium azide. This ring-opening reaction seems to be completely stereo- and regioselective. **Rac-4c** was isolated in 74% yield after purification (scheme 4).

Results and Discussion

An enzyme screening was used on each substrate in order to find out which of the tested enzymes is suited best for a kinetic resolution of a certain substrate. The enzymes tested were porcine pancreatic lipase, lipases from *Candida rugosa* (CRL), *Pseudomonas cepacia* (PCL), *Geotrichum candidum* (GCL) and *Mucor javanicus* and the proteases α -chymotrypsin and papain.

CRL was active for all three substrates, GCL showed moderate activity only for substrate **2**, while PCL was only active for substrate **3**. This is in accordance with results from other authors dealing with the size of the active site of these lipases¹⁹.

For the enzymatic resolution the reaction was terminated at a conversion of about 40%. The product was isolated and the remaining substrate was subjected to a second hydrolytic step up to an overall conversion of about 60%. The course of hydrolysis was monitored by consumption of 1N NaOH. The alcohol of the nonconverted ester was obtained by methanolysis with catalytic amounts of sodium methylate. Results of the kinetic resolutions are summarized in table 1.

Table 1 Results of the kinetic resolution of substrates **2-4**

Subst.	Enz.	Hydrolyzed Alcohol						Remaining Ester			
		time (h)	conv. ^a (%)	$[\alpha]_D^{20}$ ^b	Isom. ^c	e.e. ^d (%)	E*	conv. ^a (%)	$[\alpha]_D^{20}$ ^b	Isom. ^c	e.e. ^d (%)
2	CRL	1	15f	-20.8	1R,4R	>98	>100	69	+29.0	1S,4S	>98
2	GCL	22	13f	-20.7	1R,4R	>98	>100	61	+21.1	1S,4S	70
3	CRL	2	19f	-34.8	1R,3R	95	48	50	+19.3	1S,3S	51
3	PCL	30	11f	+15.5	1S,3S	74	7	89	-0.4	1R,3R	n.d.
4	CRL	17	37	-10.9g	1R,3R	97	>100	63	+22.4	1S,3S	>98

a Measured by consumption of 1N NaOH (calculated for diol)

b In acetone-solution (c = 2), at 20°C

c Estimated absolute configuration of the isomer

d Determined by ¹⁹F-nmr of the (S)-MTPA-ester

e $E = \ln [1 - c(1 + e.e._p)] / \ln [1 - c(1 - e.e._p)]$; P = product

f The conversion to the diol is so low, because the reaction was stopped after 40% absolute conversion of one ester unit

g In CH₂Cl₂-solution (c = 2)

By enzymatic hydrolysis of **rac-2** and **-3**, firstly monoesters **2d** and **3d** are formed. Unfortunately the reaction is not as selective, as to stop at this stage. So there were always diol, mono- and diester in the reaction mixture. After 22 hours with GCL as catalyst for **rac-2**, 28% of the diester was hydrolyzed to monoester and 46% of the built monoester was hydrolyzed to give diol. So we got an overall yield of 13% diol and 15% monoester. The diol formed was (1R,2R,4R,5R)-2,5-diazido-1,4-cyclohexanediol **2c** and the monoester (1R,2R,4R,5R)-4-butanoyloxy-2,5-diazidocyclohexanol **2d**.

Relative configurations were determined by ^1H -nmr and absolute configurations assigned by comparison of specific rotations with known compounds of similar nature. CRL was very active for **rac-2**. Already after one hour, 35% of the diester have been hydrolyzed and 43% of the monoester was hydrolyzed to diol, so we got 15% diol with over 98% enantiomeric excess. GCL and CRL showed the same enantioselectivity for **rac-2**, and both are highly selective. PCL showed no activity for substrate **rac-2**, while it was active in the case of substrate **rac-3** although not very selective. The enantioselectivity was opposite to CRL, which was also very reactive for **rac-3**. While PCL hydrolyzed the ester group with the *S*-configuration, CRL hydrolyzed the *R*-configured ester. For substrate **rac-4**, CRL was the only active lipase. The reactivity of CRL for **rac-4** compared to **rac-2** and **rac-3** was much lower, while the selectivity remained high. In the case of **rac-4**, the enzymatic hydrolysis to monoester was proved to be highly selective and only the ester function at C-1 was hydrolyzed. The monoester is very unstable and during work-up it was partly hydrolyzed just by water to the diol, which was not observed during the enzymatic hydrolysis. As no asymmetric step was involved in the formation of the diol, the enantioselectivity remained untouched. It was not surprising that the ester at C-1 was hydrolyzed, because the ester function at C-3 is sterically shielded by the two neighbouring azido groups, so the enzyme might encounter difficulties in forming the ES-complex. As expected, PCL and GCL could not hydrolyze **rac-4**, because of the small active site pockets¹⁹.

The investigations into substrates **2**, **3** and **4** show exactly that the lipases are much more flexible than first accepted. They are very useful tools for kinetic resolutions of complex and highly functionalized cyclic molecules. The azido group has proved to be a very suitable binding group^{11a,b}, but if the ester group is buried between two azido groups, the lipase apparently cannot accept it. This is a further proof of the active site models for these lipases²² and the recently published x-ray structures of CRL^{19d,e}, PCL^{19f} and GCL^{19c}.

Experimental

Optical rotations were measured on a JASCO DIP-370 polarimeter. Nmr spectra were recorded on a Bruker MSL 300 at 300.13 MHz (^1H), 75.47 MHz (^{13}C), and 282.2 MHz (^{19}F) or on a VARIAN Gemini 200 at 199.98 MHz (^1H) and 50.29 (^{13}C), respectively. Chemical shifts are given in ppm downfield relative to TMS or CFCl_3 as internal standard, s = singlet, d = doublet, t = triplet, q = quadruplet, hex = hextet, m = multiplet. In the numbering of carbon atoms in nmr data, prime (') denotes carbons in the butyric acid part. Reactions were monitored by TLC using silica gel Merck 60F₂₅₄ plates, purification of products and separation of esters and alcohols after enzymatic conversions were performed on silica gel Merck 60 with mixtures of ethyl acetate and light petroleum or ethyl acetate and cyclohexane as mobile phase. All commercially available compounds were used as received. Crude enzyme preparations were employed without further purification. PCL (13.000 U/mg) and GCL are donations from Amano Pharmaceutical Co., CRL (10 U/mg) was purchased from Sigma (1U is able to liberate 1 μmole fatty acid/min at pH 7.0 and 25°C).

The enantiomeric excesses after enzymatic resolutions were determined by ^{19}F -nmr of the respective (*S*)-MTPA (α -methoxy- α -trifluoromethylphenyl acetic acid) esters²⁰.

General procedure for esterification with butyric anhydride²¹:

The respective alcohol (**rac-2c**, **-3c**, **-4c**) (10.0 mmoles) was dissolved in 50 ml absolute methylene chloride, butyric anhydride (Merck) (26.0 mmoles), absolute pyridine (30.0 mmoles) and a catalytic amount of *N,N*-dimethylamino pyridine (DMAP) were added. Usually after 16 hours, the alcohol was converted to the ester. To stop the reaction, 1 ml MeOH was added and the solution stirred for about 30 minutes. The transparent solution was extracted with aqueous 10% HCl, saturated aqueous NaHCO_3 , and water. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude products were purified by chromatography. The yields were usually >90%. Nmr-data (CDCl_3) of hitherto unknown substrates for enzymatic hydrolyses (assignments in most cases by 2D-experiments):

(1r, 2t, 4c, 5f)-2,5-diazido-1,4-dibutanoyloxycyclohexane (rac-2)

δ_{H} 0.94 (6H, t, 2x 4'-H); 1.65 (4H, hex, 2x 3'-H); 1.96 (4H, m, 3- and 6-H); 2.31 (4H, t, 2 x 2'-H); 3.75 (2H, q, 2- and 5-H, $J = 4.4$ Hz); 4.95 (2H, q, 1- and 4-H, $J = 4.1$ Hz).

δ_{C} 13.6 (2 x C-4'); 18.4 (2 x C-3'); 30.1 (C-3, C-6); 36.2 (2 x C-2'); 58.5 (C-2, C-5); 69.8 (C-1, C-4); 172.3 (2 x C-1').

(1r, 3t, 4c, 6f)-4,6-diazido-1,3-dibutanoylcyclohexane (rac-3)

δ_{H} 0.91 (6H, t, 2 x 4'-H); 1.62 (4H, hex, 2 x 3'-H); 1.91 (2H, t, 5-H, $J = 5.9$ Hz); 1.95 (2H, t, 2-H, $J = 5.6$ Hz); 2.28 (4H, t, 2 x 2'-H); 3.75 (2H, q, 4- and 6-H, $J = 6.2$ Hz); 4.94 (2H, q, 1- and 3-H, $J = 6.2$ Hz).

δ_{C} 13.6 (2 x C-4'); 18.3 (2 x C-3'); 30.0 (C-5); 30.3 (C-2); 36.1 (2 x C-2'); 58.1 (C-4, C-6); 70.2 (C-1, C-3); 172.4 (2 x C-1').

***trans*-3,8-dioxatricyclo[5.1.0.0^{2,4}]octane (*trans*-4b)**

δ_{H} 1.70 (4H, br, 2- and 3-H); 3.15 (2H, dt, 1- and 4-H, $J_{1,2}=J_{3,4} = 1.8$ Hz, $J_{1,7}=J_{4,6} = 1.7$ Hz); 3.34 (2H, dd, 6- and 7-H, $J_{1,7}=J_{4,6} = 1.6$ Hz, $J_{6,7} = 3.6$ Hz)

δ_{C} 19.1 (C-2, C-3); 50.1 (C-1, C-4); 53.2 (C-6, C-7).

(1r, 2t, 3t, 4c)-2,4-diazido-1,3-cyclohexanediol (rac-4c)

δ_{H} 1.62-1.96 (4H, m, 5- and 6-H); 3.73 (2H, m, 2- and 4-H); 4.01 (2H, 1- and 3-H); 4.19 (2H, br, 2x -OH).

δ_{C} 23.4 (C-5); 27.4 (C-6); 61.3 (C-4); 66.5 (C-2); 69.1 (C-1); 71.4 (C-3).

(1r, 2t, 3t, 4c)-2,4-diazido-1,3-dibutanoyloxycyclohexane (rac-4)

δ_{H}	0.94 (3H, t, 4'-H); 0.95 (3H, t, 4'-H); 1.52-1.97 (8H, m, 2 x 3'-H, 5- and 6-H); 2.31 (2H, t, 2'-H); 2.37 (2H, t, 2'-H); 3.80 (1H, dt, 4-H, $J_{4a,3a} = J_{4a,5a} = 8.2$ Hz, $J_{4a,5c} = 4.0$ Hz); 3.93 (1H, dd, 2-H, $J_{2c,1c} = 5.8$ Hz, $J_{2c,3a} = 3.3$ Hz); 5.00 (1H, dt, 1-H, $J_{1c,2c} = J_{1c,6c} = 5.8$ Hz, $J_{1c,6a} = 2.9$ Hz); 5.09 (1H, dd, 3-H, $J_{3a,2c} = 3.4$ Hz, $J_{3a,4a} = 8.3$ Hz).
δ_{C}	13.6 (2 x C-4'); 18.2 (C-3'); 18.4 (C-3'); 23.7 (C-5); 24.5 (C-6); 35.9 (C-2'); 36.2 (C-2'); 58.2 (C-4); 61.0 (C-2); 70.0 (C-1) 72.9 (C-3); 172.4 (2 x C-1').

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References

1. Moellering Jr., R.C. in: *The Amino glycosides: Microbiology, Clinical Use and Toxicology*, Whelton, A.; Neu, H.C. (Eds.) Marcel Dekker, Inc. New York, Basel 1982, 65-95.
2. Naghabhushan, T.L.; Miller, G.H.; Weinstein, M.J. in: *The Amino glycosides: Microbiology, Clinical Use and Toxicology*, Whelton, A.; Neu, H.C. (Eds.) Marcel Dekker, Inc. New York, Basel 1982, 3-27.
3. Nara, T. *J. Antibiot.* **1977**, *30*, 533-540.
4. Kobayashi, S.; Kamiyama, K.; Ohno, M. *Chem. Pharm. Bull.* **1990**, *38*, 350-354.
5. Miyake, T.; Tsuchiya, T.; Umezawa, S.; Umezawa, H. *Carbohydr. Res.* **1976**, *49*, 141-151.
6. Leitner, F.; Price, K. in: *The Amino glycosides: Microbiology, Clinical Use and Toxicology*, Whelton, A.; Neu, H.C. (Eds.) Marcel Dekker, Inc. New York, Basel 1982, 29-64.
7. a: Liu, P.; Vandenwalle, M. *Tetrahedron Lett.* **1993**, *34*, 3625-3628. b: Knapp, S.; Sebastian, M. J.; Ramanathan, H.; Bharadwaj, P.; Potenza, J. A. *Tetrahedron* **1986**, *42*, 3405-3410. c: Kühlmeyer, R.; Schwesinger, R.; Prinzbach, H. *Tetrahedron Lett.* **1984**, *25*, 3429-3432. d: Schubert, J.; Schwesinger, R.; Knothe, L.; Prinzbach, H. *Liebigs Ann. Chem.* **1986**, 2009-2052.
8. Kobayashi, S.; Kamiyama, K.; Ohno, M. *J. Org. Chem.* **1990**, *55*, 1169-77.
9. a: Castellanos, L.; Cleophax, J.; Colas, C.; Gero, S. D.; Leboul, J.; Mercier, D.; Olesker, A.; Rolland, A.; Quilet-Sire, B.; Sepulchre, A.-M. *Carbohydr. Res.* **1980**, *82*, 283-301. b: Dureault, A.; Greck, C.; Depezay, J.-C.; *J. Carbohydr. Chem.* **1990**, *9*, 121-3. c: Honda, Y.; Suami, T. *Bull. Chem. Soc. Jpn.*, **1982**, *55*, 1156-1162. d: Sano, H.; Sakaguchi, T.; Mori, Y. *Bull. Chem. Soc. Jpn.*, **1979**, *52*, 2727-2728.
10. Carless, H.A.J.; Malik, S.S. *Tetrahedron: Asymmetry* **1992**, *3*, 1135-1138.
11. a: Gruber-Khadjawi, M.; Hönlig, H. *Biocatalysis* **1994**, *9*, 249-259. b: Gruber-Khadjawi, M.; Hönlig, H.; Weber, H. *Chirality* **1992**, *4*, 103-109. c: Hönlig, H.; Seuffer-Wasserthal, P. *Synthesis* **1990**, 1137-1140. d: Hönlig, H.; Seuffer-Wasserthal, P.; Stütz, A.E.; Zenz, E. *Tetrahedron Lett.* **1989**, *30*, 811-812. e: Hönlig, H.; Seuffer-Wasserthal, P.; Weber, H. *Tetrahedron* **1990**, *46*, 3841-3850. f: Foelsche, E.; Hickel, A.; Hönlig, H.; Seuffer-Wasserthal, P. *J. Org. Chem.*, **1990**, *55*, 1749-1753.

12. Presented at the 11. Scientific Meeting of the Austrian Pharmaceutical Society, 7.-9. 4. 1994 in Graz, Austria.
13. Presented at the 8. Austrian Chemical Society Meeting, 28.-30. 9. 1994 in Graz, Austria.
14. Kavadias, G.; Velkof, S.; Belleau, B. *Can. J. Chem.*, **1978**, *56*, 404-409.
15. Craig, T.W.; Harvey G.P.; Berchtold, G.A. *J. Org. Chem.*, **1967**, *32*, 3743-3749.
16. Kavadias, G.; Belleau, B. **1977**, U.S. Patent, 12 pp., US 4003922.
17. McCasland, G.E.; Anisuzzaman, A.K.M.; Naik, S.R.; Durham, L.J. *J. Org. Chem.*, **1972**, *37*, 1201-1204.
18. a: Suemune, H.; Hasegawa, A.; Sakai, K. *Tetrahedron: Asymmetry* **1995**, *6*, 55-58. b: Ramanathan, J.D.; Craigie, J.S.; McLchlan, J.; Smith, D.G.; McInnes, A.G. *Tetrahedron Lett.* **1966**, *14*, 1527-
c: Hönig, H.; Seuffer-Wasserthal, P.; Fülöp, F. *J. Chem. Soc. Perkin Trans. 1*, **1989**, 2341-2345.
19. a: Faber, K. in: Biotransformations in Organic Chemistry, Springer Verlag, Berlin, Heidelberg, New York, 2nd. Edition **1995**. b: Exl, C.; Hönig, H.; Renner, G.; Rogi-Kohlenprath, R.; Seebacher, V.; Seuffer-Wasserthal, P. *Tetrahedron: Asymmetry* **1992**, *3*, 1391-1394. c: Schrag, J.D.; Li, Y.; Wu, S.; Cygler, M.; *Nature*, **1991**, *351*, 761-764. d: Longhi, S.; Lotti, M.; Fusetti, F.; Pizzi, E.; Tramontano, A.; Alberghina L. *Biochim. Biophys. Acta* **1992**, *1165*, 129-133. e: Grochulski, P.; Schrag, J.D.; Bouthillier, F.; Smith, P.; Harrison, D.; Rubin, B.; Cygler, M. *J. Biol. Chem.* **1993**, *268*, 12843-12847. f: Noble, M.E.M.; Cleasby, A.; Johnson, L.N.; Egmond, M.R.; Frenken, L.G.J. *FEBS Lett.*, **1993**, *331*, 123-128.
20. Sullivan, G.R.; Dale, J.A.; Mosher, H.S. *J. Org. Chem.*, **1973**, *38*, 2143-2147.
21. Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem.*, **1978**, *90*, 602-615.
22. a: Bhalerao, U.T.; Dasaradhi, L.; Neelakantan, P.; Fadnavis, N.W. *J. Chem. Soc., Chem. Commun.*, **1991**, 1197-1198. b: Xie, Z.-F. *Tetrahedron: Asymmetry* **1991**, *2*, 733-750. c: Xie, Z.-F.; Nakamura, I.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.*, **1988**, 966-967.

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