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

Mandana Gruber-Khadjawi, Helmut Hönig\* and Carina Illaszewicz

Institute of Organic Chemistry, Technical University of Graz, Stremayrgasse 16, A-8010 Graz, AUSTRIA

Abstract: Optically active 2,5-diazido-1,4-cyclohexanediol, 4,6-diazido-1,3-cyclohexanediol and 2,5diazido-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 moiety<sup>1</sup>. The aminoglycoside antibiotics are the oldest known class of broad-spectrum antibacterials with activity against grampositive and gram-negative organisms as well as mycobacteria. They are, however, not active against anaerobic bacteria or fungi<sup>2</sup>.

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*<sup>3</sup>, all carbons of the cyclohexane ring are asymmetric, being substituted with oxygen or nitrogen functional groups<sup>4</sup>. 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 activity<sup>5</sup>. 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 enzymes<sup>6</sup>. In the literature the synthesis of chiral fortamines are already described<sup>7</sup>. In 1990 Kobayashi reported the first enantioselective synthesis of fortamine using pig liver esterase as chiral catalyst<sup>4,8</sup>, while other groups used the chiral pool<sup>9</sup>. In 1992 Carless and collaborators described the enantiospecific synthesis by microbial oxidation of toluene<sup>10</sup>.



In continuation of our earlier work<sup>11</sup>, 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 antibiotica), and 2,4-diazido-1,3-cyclo-hexanediol  $4^{13}$  (derivative of 5,6-dideoxystreptamine).



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<sup>14,16</sup>. 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<sup>15,17</sup> (scheme 3). The absolute configuration after the kinetic resolution step was estimated by comparison of the specific rotation values with similiar known compounds<sup>18</sup>.





Scheme 4



a) CH\_3COOOH/ CH\_3COONa/ Na\_2CO\_3/ CH\_2Cl\_2 b) NaN\_3/ NH\_4Cl / EVDH-water c) (C\_3H\_7CO)\_2O/ pyridine/ DMAP/ CH\_2Cl\_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 ringopening 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 porcin pancreatic lipase, lipases from *Candida rugosa* (CRL), *Pseudomonas cepacia* (PCL), *Geotrichum candidum* (GCL) and *Mucor javanicus* and the proteases  $\alpha$ -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 lipases<sup>19</sup>.

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

		Hydrolyzed Alcohol						Remaining Ester			
Subst.	Enz.	time (h)	conv." (%)	[α] <sub>0</sub> Þ	lsom.º	e.e. <sup>d</sup> (%)	E•	conv.* (%)	[α] <sub>0</sub> <sup>5</sup>	lsom.º	e.e." (%)
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 <sup>19</sup>F-nmr of the (S)-MTPA-ester

 $E = ln [1 - c (1 + e.e._{p})] / ln [1 - c (1 - e.e._{p})]; P = product$ 

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

g In  $CH_2CI_2$ -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-2,5diazidocyclohexanol 2d. Relative configurations were determined by <sup>1</sup>H-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 enantiopreference 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 enantiopreference 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*-configurated 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 pockets<sup>19</sup>.

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<sup>11a,b</sup>, 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 lipases<sup>22</sup> and the recently published x-ray structures of CRL<sup>19d,e</sup>, PCL<sup>19f</sup> and GCL<sup>19c</sup>.

#### Experimental

Optical rotations were measured on a JASCO DIP-370 polarimeter. Nmr spectra were recorded on a Bruker MSL 300 at 300.13 MHz (<sup>1</sup>H), 75.47 MHz (<sup>13</sup>C), and 282.2 MHz (<sup>19</sup>F) or on a VARIAN Gemini 200 at 199.98 MHz (<sup>1</sup>H) and 50.29 (<sup>13</sup>C), respectively. Chemical shifts are given in ppm downfield relative to TMS or CFCl<sub>3</sub> 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  $60F_{254}$  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 1µmole fatty acid/min at pH 7.0 and 25°C).

The enantiomeric excesses after enzymatic resolutions were determined by <sup>19</sup>F-nmr of the respective (S)-MTPA ( $\alpha$ -methoxy- $\alpha$ -trifluoromethylphenyl acetic acid) esters<sup>20</sup>.

General procedure for esterification with butyric anhydride<sup>21</sup>:

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<sub>3</sub>, and water. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent removed under reduced pressure. The crude products were purified by chromatography. The yields were usually >90%. Nmr-data (CDCl<sub>3</sub>) of hitherto unknown substrates for enzymatic hydrolyses (assignments in most cases by 2D-experiments):

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

- $\delta_{\rm H}$  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<sub>c</sub> 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, 6t)-4,6-diazido-1,3-dibutanoylcyclohexane (rac-3)

- $\delta_{\rm H}$  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<sub>c</sub> 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<sup>2,4</sup>]octane (trans-4b)

- $\delta_{\rm H}$  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)
- $\delta_{\rm C}$  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<sub>H</sub> 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).
- $\delta_{\rm C}$  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)

- $$\begin{split} \delta_{\rm H} & 0.94 \; (3{\rm H}, \, t, \, 4'{\rm -H}); \; 0.95 \; (3{\rm H}, \, t, \, 4'{\rm -H}); \; 1.52{\rm -}197 \; (8{\rm H}, \, m, \, 2 \, {\rm x} \; 3'{\rm -H}, \; 5{\rm -} \; \text{and} \; 6{\rm -H}); \; 2.31 \; (2{\rm H}, \, t, \, 2'{\rm -H}); \\ 2.37 \; (2{\rm H}, \, t, \, 2'{\rm -H}); \; 3.80 \; (1{\rm H}, \, {\rm dt}, \; 4{\rm -H}, \, J_{4a,3a} = J_{4a,5a} = 8.2 \; {\rm Hz}, \; J_{4a,5c} = 4.0 \; {\rm Hz}); \; 3.93 \; (1{\rm H}, \; {\rm dd}, \; 2{\rm -H}, \, J_{2c,1c} = 5.8 \; {\rm Hz}, \; J_{2c,3a} = 3.3 \; {\rm Hz}); \; 5.00 \; (1{\rm H}, \; {\rm dt}, \; 1{\rm -H}, \, J_{1c,2c} = J_{1c,6c} = 5.8 \; {\rm Hz}, \; J_{1c,6a} = 2.9 \; {\rm Hz}); \; 5.09 \; (1{\rm H}, \; {\rm dd}, \; 3{\rm -H}, \; J_{3a,2c} = 3.4 \; {\rm Hz}, \; J_{3a,4a} = 8.3 \; {\rm Hz}). \end{split}$$
- δ<sub>c</sub> 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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