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Microbial Baeyer–Villiger Reaction of Bicyclo[3.2.0]heptan-6-ones — A Novel Approach to Sarkomycin A

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Abstract—Racemic (1α,2α,5α)- and (1β,2α,5β)-2-bromobicyclo[3.2.0]heptan-6-one (*rac*-**7**, *rac*-**10**, respectively), (1α,2α,5β)- and (1β,2α,5β)-2-benzyloxybicyclo[3.2.0]heptan-6-one (*rac*-**15**, *rac*-**13**, respectively), (1β,2α,5β)-2-hydroxybicyclo[3.2.0]heptan-6-one (*rac*-**17**) and *cis*-bicyclo[3.2.0]hept-2-en-7-one (*rac*-**18**) were subjected to a microbial Baeyer–Villiger reaction by *Acinetobacter calcoaceticus* NCIB 9871. In each case both regioisomeric lactones were formed (67–93 % yield) having always the opposite configuration (20 to > 99 % *e.e.*). Both the ratio of the regioisomers and the enantiomeric excess proved to be dependent on the type of substitution. Analogously *cis*-bicyclo[3.2.0.]heptan-2,6-dione (*rac*-**1**) gave besides other products cyclosarkomycin (**1b**) (7 % yield, 97 % *e.e.*). Compound **1b** was also obtained from the Baeyer–Villiger product of *rac*-**17** by Swern oxidation (total yield starting from *rac*-**17** 9 %, > 98 % *e.e.*).

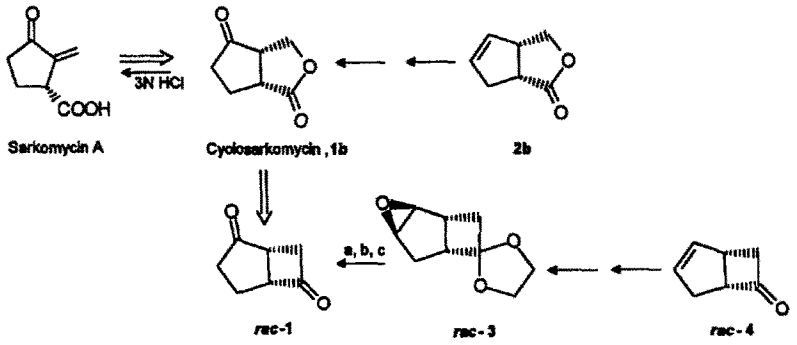
Introduction

Due to its biological properties as an antitumor, antiviral and antibacterial agent the antibiotic sarkomycin[4] has been the target of numerous synthetic efforts.[5] Due to its instability it is preferable to choose as target molecule for the preparation of the most important member of this group, sarkomycin A, the stable precursor cyclosarkomycin (**1b**) which can be converted into sarkomycin A by treatment with acid.[5c,6] One of the starting materials used for a synthesis of racemic **1b** was lactone **2b**.[6b] However, the regioselective functionalization of the double bond proved to be difficult. Since lactones of this structural type can be obtained enantioselectively by microbial Baeyer–Villiger reaction of racemic starting material[7] and in continuation of our prior work in this field[8] the biocatalytic transformation of several substituted bicyclo[3.2.0]heptan-6-ones and of bicyclo[3.2.0]heptan-2,6-dione has been investigated.

Results

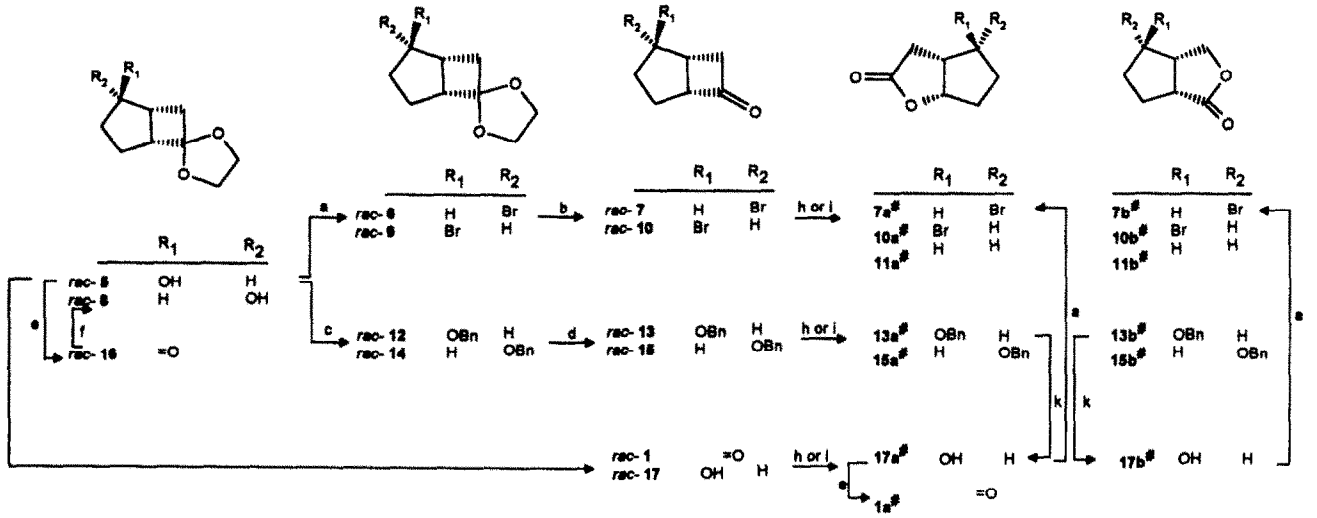
Synthesis of starting materials

In Schemes I and II the syntheses are shown for those bicyclic ketones which were subjected to both the microbial Baeyer–Villiger reaction, and also the corresponding non-biological chemical transformation for comparison purposes. Starting point was known epoxide *rac*-**3**, easily obtained by cycloaddition of dichloroketene to 1,3-cyclopentadiene, reductive removal of the chlorine atoms followed by protection and epoxidation.[9] The epoxide was regioselectively opened with lithium aluminium hydride to give *rac*-**5** followed by Swern oxidation[10] to the partially protected bicyclic ketone *rac*-**16** from which by deprotection diketone *rac*-**1** was synthesized.



Scheme I.[2] Synthetic pathways to sarkomycin. (a) $LiAlH_4$/THF. (b) Oxalyl chloride/DMSO, CH_2Cl_2, Et_3N. (c) Acetic acid (30 %), 70 °C, 1 h.

Dedicated to Professor J. Bryan Jones in honour of his 60th birthday.



Scheme II.[2] Chemical and microbial transformations. By chemical Baeyer–Villiger reaction (conditions h) all compounds marked with '#' are formed as racemic mixtures. (a) $Ph_3P/Br_2/Et_3N$. (b) Acetic acid (30 %), 70 °C, 1 h. (c) NaH/benzyl bromide/THF. (d) H_2SO_4, acetone, room temp. (e) Oxalyl chloride/DMSO, CH_2Cl_2, Et_3N. (f) $NaBH_4$, CH_3OH, –20 °C. (g) Acetic acid (30 %), 80 °C, 1 h. (h) MCPBA/$NaHCO_3$, CH_2Cl_2. (i) *Acinetobacter calcoaceticus* NCIB 9871. (k) H_2, 10 % Pd/C, CH_3OH.

To obtain the other substrates *rac*-**16** was reduced to the all-*cis* bicyclic alcohol *rac*-**8** using sodium tetrahydroborate in methanol at –20 °C. Both diastereomers *rac*-**5** and *rac*-**8** were then subjected to bromination by triphenylphosphine/bromine/triethylamine[11] to give diastereomeric bromides *rac*-**6** and *rac*-**9** and after deprotective hydrolysis bromoketones *rac*-**7** and *rac*-**10**, respectively. Benzyloxyketones *rac*-**13** and *rac*-**15** were synthesized analogously by reaction of *rac*-**5** and *rac*-**8** with benzyl bromide/sodium hydride/THF followed by deprotection. Ketone *rac*-**17** was obtained from the protected precursor *rac*-**5** by hydrolysis, ketone *rac*-**18** was synthesized as described.[12]

Baeyer–Villiger reactions

In Table 1 the results of both chemical and microbial reactions are given. The chemical oxidation[16] was performed using either hydrogen peroxide/90 % acetic acid for substrate *rac*-**18**[19] or *meta*-chloroperbenzoic acid/sodium hydrogen carbonate for the transformation of the other starting compounds. As expected, the main products were lactones of the 2-oxabicyclo[3.3.0]octan-3-one type (*rac*-**7a**, *rac*-**10a**, *rac*-**13a**, *rac*-**15a**, *rac*-**17a** and *rac*-**18a**). Diketone *rac*-**1** did not react under the conditions given above. Therefore, lactone *rac*-**17a** was oxidized using the Swern procedure[10] to provide reference material.

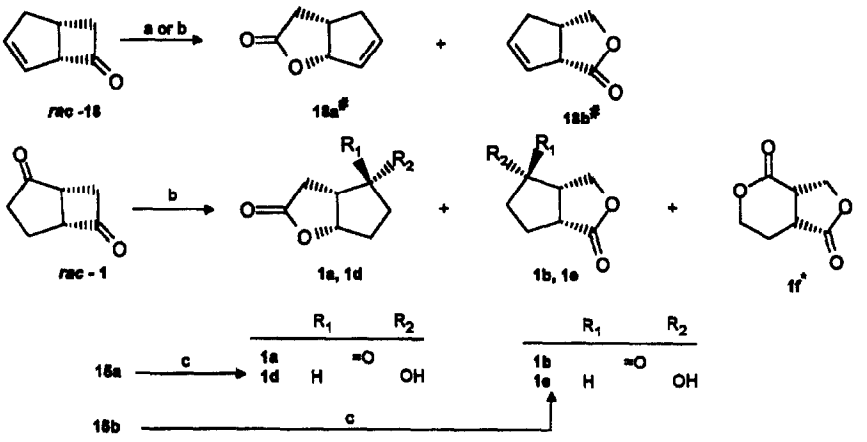
For the microbial transformation *Acinetobacter calcoaceticus* NCIB 9871 was employed which had proven to give enantioselective Baeyer–Villiger reaction of bicyclic ketones; the experimental procedure reported in the literature was followed in principle.[7] Contrary to the chemical reaction both possible Baeyer–Villiger products were obtained, as a rule in nearly equal amounts. These diastereomeric lactones were, as expected,[7] of opposite absolute configuration. In most cases the enantiomeric excess achieved was high. When diketone *rac*-**1** was subjected to this biotransformation the Baeyer–Villiger product from the cyclobutenone moiety was formed in 36 % yield. Twenty per cent of this product material was cyclosarkomycin (**1b**, 97 % *e.e.*) which was isolated by column chromatography (see Scheme III). The other products formed were the enantiomeric hydroxylactones **1d** and **1e** which originated from an enzymatic reduction of **1a** and **1b** during the biotransformation, plus dilactone **1f*** which originated from Baeyer–Villiger reaction of both keto groups of the starting material. For compound **1f*** neither the enantiomeric composition nor the absolute configuration were determined. The mixture of hydroxylactones **17a** and **17b** produced by subsequent Swern oxidation and chromatographic separation again furnished cyclosarkomycin (**1b**), thus raising an alternative approach to this compound (total yield starting from *rac*-**17** 32 %, 98 % *e.e.*).

Except for lactones **1a/1b** and **18a/18b** the chromatographic separation proved to be difficult. Therefore, for determination of optical purity, lactones **13a/13b** and **15a/15b** were debenzylated by hydrogenolysis followed by bromination[11] of the product alcohols to give bromolactones **7a/7b** and **10a/10b**, respectively, the enantiomers of which could be separated by gas chromatography using a column with permethyl-β-cyclodextrin in OV 1701 as the stationary phase. This column was also used for the determination of the enantiomeric excess in lactones **1a/1b** and **18a/18b**. For compounds **1d/1e** and **17a/17b** the enantiomeric excess was measured analogously to the procedure used for **13a/13b**.

For the determination of absolute configuration bromolactones **7a/7b** and **10a/10b** were debrominated using tributyltin hydride and AIBN in benzene to give

Table 1. Chemical and microbial Baeyer–Villiger reactions

	Chemical Baeyer-Villiger reaction		Microbial Baeyer-Villiger reaction	
Substrate	Products	Yield	Products	Yield
rac-7	95% *rac* - 7a + 5% *rac* - 7b	86%	66% 7a (48% e.e.) + 34% 7b (97% e.e.)	93%
rac-10	97% *rac* - 10a + 3% *rac* - 10b	83%	62% 10a (54% e.e.) + 38% 10b (95% e.e.)	79%
rac-13	92% *rac* - 13a + 8% *rac* - 13b	69%	54% 13a (44% e.e.) + 46% 13b (78% e.e.)	71%
rac-15	94% *rac* - 15a + 6% *rac* - 15b	81%	32% *rac* - 15a + 68% 15b(20% e.e.)	78%
rac-17	96% *rac* - 17a + 4% *rac* - 17b	56%	52% 17a (90% e.e.) + 48% 17b (>98% e.e.)	75%
rac-18	95% *rac* - 18a + 5% *rac* - 18b	75%	52% 18a (96% e.e.) + 48% 18b (>99% e.e)	67%
rac-1			79% 1a(92% e.e.) + 21% 1b (97% e.e.)	36%
			21% 1d (90% e.e.) + 25% 1e (>99% e.e.) + 54% 1f	28%



Scheme III.[2,3] Chemical and microbial transformations, continued. By chemical Baeyer–Villiger reaction (conditions a) all compounds marked with '#' are formed as racemic mixtures. (a) Acetic acid (90 %)/H_2O_2, 0 °C. (b) *Acinetobacter calcoaceticus* NCIB 9871. (c) H_2; 10 % Pd/C, CH_3OH.

lactones **11a/11b** the enantiomeric composition of which was investigated by gas chromatography as above. For comparison enantiomerically enriched **11a** (with 1*R*,5*R*-configuration) was synthesized by catalytic hydrogenation (H_2, 5 % Pd/C) of enantiomerically enriched **18a**[13] obtained from (2*S*, 4*S*)-norborn-5-en-2-one.[14] Analogously lactone **11b** (1*R*,5*S*-configuration already established[15]) was prepared by hydrogenation of the unsaturated compound **18b**.

Discussion

In general the results of the Baeyer–Villiger reactions investigated in this study are in accordance with previous reports with respect to the regiochemistry of product formation.[7] However, contrary to the chemical reaction where the regioselection observed was nearly independent of the substituents present in the substrate, in the microbiological experiments a strong influence by the substitution pattern is observed (see Table 1). The two regioisomeric lactones are formed with opposite absolute configuration. The enantiomeric excess achieved is dependent on both size and relative configuration of the substituents investigated. In the case of the space-filling benzyloxy group (ketones *rac*-**13** and *rac*-**15**) not only is the degree of enantioselectivity reduced, but reversed regioselectivity is also observed for substrate *rac*-**15**. In an attempt to determine the enantiomeric composition of benzyloxylactone **15a** formed by the microbial reaction this compound was hydrogenated to give hydroxylactone **17a** followed by transformation into bromolactone **7a**. Surprisingly, this was found to be racemic. The influence of the size of substituents on the enantioselectivity of the microbial Baeyer–Villiger reaction fits[17] into the models discussed at present for the respective monooxygenases of *A. calcoaceticus*.[7,18]

Regarding the synthetic aspects two novel pathways to highly enantiopure cyclosarkomycin have been developed: either from the easily accessible *rac*-**1**[19] by *A. calcoaceticus* in 7 % yield (97 % *e.e.*) or by the same microorganism from hydroxyketone *rac*-**17**, prepared in 59 % yield from epoxide *rac*-**3**,[9] thus furnishing hydroxylactone **17b** in 36 % yield (> 98 % *e.e.*) which is then oxidized to cyclosarkomycin (total yield starting from *rac*-**3** 5 %).

Experimental Section

^1H NMR Spectra have been obtained as a rule if not otherwise stated in $CDCl_3$ solvent on a Bruker MSL300 (300 MHz) spectrometer or a Bruker AW200 (200 MHz) spectrometer. Chemical shifts are given in δ (ppm), TMS as internal standard. ^{13}C NMR Spectra have been obtained on a Bruker MSL300 (75.47 MHz) in $CDCl_3$. Chemical shifts are given in δ (ppm) relative to $CDCl_3$ (77.27 ppm). As a rule these spectra were decoupled. Otherwise C-H multiplicities are given in parentheses. For preparative gas chromatography Shimadzu GC 14A with hot wire detector was used, column: 3.6 m × 3 mm packed with 10 % SP 2100 (methyl silicone) on Supelcoport 100/120, carrier gas: helium. For thin layer chromatography aluminium foils coated with silica gel 60 F_{254} (Merck) were used, detection mainly by spraying with a 5 % solution of vanillin in concentrated sulphuric acid ('VS'). The following eluents have been used: A: petroleum ether bp 60–80 °C/ethyl acetate 10:1; B: the same 3:1; C: the same 1:1; D: the same 1:3; E: chloroform/methanol 19:1. Optical rotations have been determined in a Jasco DIP 370 polarimeter. For analytical gas chromatographic investigations (FID) the following columns have been used: column C: Biorad RSL1701, 30 m × 0.25 mm × 0.25 μm (polydimethyl siloxane, 14 % cyanopropyl-phenylsiloxane); column D: J&W Cyclodex B, 30 m × 0.25 mm (permethyl-β-cyclodextrin in OV1701).

General procedures

Procedure 1: Swern oxidation.[10] In a two neck flask with pressure relief and dropping funnel (or septum for small amounts, drop by drop addition with a syringe) 1.2 eq. of oxalyl chloride were dissolved in a 20-fold amount of anhydrous (w/w) CH_2Cl_2 under N_2-atmosphere and cooled to –70 °C. Anhydrous DMSO (1.5 eq.), dissolved in a 10-fold amount of anhydrous CH_2Cl_2 (w/w) was added dropwise. The mixture was warmed to –50 °C and stirred until no more gas developed. One equivalent of the starting material dissolved in anhydrous CH_2Cl_2 is added drop by drop at –70 °C. After stirring for 15 min at –50 °C, 3 eq. of anhydrous triethylamine were added dropwise and the mixture was slowly warmed to room temperature. Extractive workup and (in most cases) chromatography gave the product.

Procedure 2: Reduction of a halide with tributyltin hydride.[23] A round bottom flask was charged with 1 eq. of halide which was dissolved in a 20-fold amount (w/w) of anhydrous benzene under N_2 atmosphere. Tributyltin hydride (1.2 eq.) and a catalytic amount of AIBN were then added. The mixture was refluxed for 1–3 h (reaction control by TLC, or better by GC) until starting material was no longer present. The solvent was removed *in vacuo* and the product was separated from the tin-compounds by extraction with acetonitrile/petroleum ether. In the acetonitrile phase the product was enriched. After removal of the acetonitrile *in vacuo* the product was purified by chromatography.

Procedure 3: Baeyer–Villiger oxidation with 3-chloroperbenzoic acid.[24] Three equivalents of anhydrous $NaHCO_3$ (powder) and small portions of 1.5 eq of 3-chloroperbenzoic acid (mCPBA) (80 %) were added to a 3 % solution of the starting material in anhydrous CH_2Cl_2. In the case of larger amounts cooling may have been necessary. A thick, white precipitate was formed. After the reaction was complete (TLC control) the mixture was washed with 10 % $NaHSO_3$ at pH 4, with saturated $NaHCO_3$ and saturated NaCl. The washing solutions were repeatedly re-extracted with CH_2Cl_2. Drying of the organic phase over Na_2SO_4 and removal of the solvent *in vacuo* gave the product which, in most cases, had to be purified by chromatography.

Procedure 4: Bromination of secondary alcohols.[11] To 1.5 eq. of triphenylphosphine in a 35-fold amount (w/w) of anhydrous CH_2Cl_2 bromine (10 % in anhydrous CH_2Cl_2) was added drop by drop until a change in color to yellow showed that the whole triphenlyphosphine had been complexed. Three equivalents of anhydrous triethylamine were added and the solution stirred for 20 min. After addition of 1.0 eq. of the secondary alcohol the reaction was stirred until no more starting material could be detected by TLC. The organic phase was washed with diluted HCl, sat. $NaHCO_3$ and the solvents removed *in vacuo*. From the semi-solid residue the product was dissolved by two extractions with ether. After removal of the ether the residue was purified by chromatography.

Procedure 5: Cleavage of an ethylene acetal.[25] The starting material and a 30-fold amount of 30 % acetic acid (w/w) were warmed to 70 °C until the reaction was complete (1–3 h, TLC or GC control). After cooling to room temperature a double volume of water was added. The solution was extracted 5 or 6 times with CH_2Cl_2, the combined organic phases were washed with saturated $NaHCO_3$ until no more acetic acid was present, then washed with saturated NaCl, dried over Na_2SO_4 and concentrated *in vacuo*.

Procedure 6: Benzylation of secondary alcohols. To a 3 % solution of the secondary alcohol in anhydrous THF 1.2 eq. of NaH were added in small portions under N_2-atmosphere. The mixture was refluxed until no more hydrogen was formed (3–12 h). After cooling to room temperature 1.3 eq. of benzyl bromide and 1 drop of HMPT were added and the mixture was heated under reflux again until the reaction was complete (TLC control). Excess benzyl bromide and eventually formed benzyl alcohol were removed by chromatography.

(1β,2α,5β)-2-Hydroxybicyclo[3.2.0]heptan-6-one ethylene acetal (rac-*5*). To 3.69 g of $LiAlH_4$ in 200 mL of anhydrous diethyl ether/THF (1:1) under an atmosphere of nitrogen was added 32.70 g (194.4 mmol) of the epoxide *rac*-**3**[9] (dissolved in the same solvent mixture) in the course of one hour. Then the mixture was refluxed until starting material was no longer (21 h) present (TLC, eluent C, VS). Then 22.2 mL of saturated aqueous $MgSO_4$ was added. The grey–white precipitate formed was removed by filtration and extracted with refluxing CH_2Cl_2. The

combined filtrates were concentrated by evaporation and purified by chromatography with 500 g of silica gel (eluent benzene/ethyl acetate 2:1). After drying *in vacuo* with an oil pump 23.27 g (136.7 mmol, 70.3 %) of a viscous liquid was isolated. ^{1}H NMR δ 1.55–2.00 (5H, m, 2 × H-3, 2 × H-4, OH); 2.25–2.45 (2H, m, 2 × H-7); 2.68 (1H, m) and 2.83 (1H, m) (H-1 and H-5); 3.47–3.90 (4H, m, O-CH_2-CH_2-O); 3.96 (1H, d(3.7 Hz), H-2). ^{13}C NMR δ 23.99; 33.70; 37.05; 37.94; 63.14 / 64.39 (OCH_2CH_2O); 77.65 (C-2); 106.58. Calcd for $C_9H_{14}O_3$: C, 63.51; H, 8.29 %; found: C, 63.57; H, 8.39 %.

cis-*Bicyclo[3.3.0]heptan-2,6-dione-6-ethylene acetal* (rac-***16***). Alcohol **5** (5.99 g, 35.2 mmol) was oxidized according to procedure 1. Chromatography (100 g of silica gel, eluent B) and drying *in vacuo* with an oil pump gave 4.50 g (26.8 mmol, 76.1 %) of a yellowish oil. ^{1}H NMR δ 1.77 (1H, ddt (13.7 Hz/9.5 Hz/9.0 Hz)); 1.92–2.13 (3H, m); 2.31–2.41 (1H, m); 2.49 (1H, dd (19.8 Hz/10.2 Hz)); 2.58 (1H, ddd (13.3 Hz/11.6 Hz/2.3 Hz)); 2.99 (1H, t (7.7 Hz)); 3.69–3.82 (4H, m, OCH_2CH_2O). ^{13}C NMR δ 20.65 (C-4); 36.25; 36.77; 39.81; 48.58; 63.07/64.65 (OCH_2CH_2O); 107.00 (C-6); 220.58 (C-2). Calcd for $C_9H_{12}O_3$: C, 64.27; H, 7.19 %; found: C, 64.40; H, 8.25 %.

cis-*Bicyclo[3.2.0]heptan-2,6-dione* (rac-***1***). Ketoacetal *rac*-**16** (3.00 g, 17.8 mmol) was hydrolysed according to procedure 5. After Kugelrohr (KR) distillation 1.83 g (14.7 mmol, 82.8 %) of the diketone *rac*-**1**[20] remained as a colorless oil. Bp(KR): 75–80 °C (0.1 kPa).

cis-*2-Oxabicyclo[3.3.0]octan-3,6-dione* (rac-***1a***). Hydroxylactone *rac*-**17a** (220 mg, 1.55 mmol) was oxidized using procedure 1. According to gas chromatography (column D) 3 % of the isomeric lactone *rac*-**1b** was also present in the product. By chromatography (8 g of silica gel, eluent C) and drying *in vacuo* with an oil pump 195 mg (1.39 mmol, 90 %) of ketolactone *rac*-**1a** were obtained, mp 58–60 °C. ^{1}H NMR δ 2.18–32 (1H, m, H-8-*endo*); 2.37–2.54 (3H, m, 2 × H-7, H-8-*exo*); 2.69 (1H, dd (17.8 Hz/1.5 Hz), H-4-*endo*) 2.84 (1H, dd (17.9 Hz/10.2 Hz), H-4-*exo*); 2.96 (1H, ddd (10.3 Hz/5.8 Hz/1.1 Hz), H-5); 5.33 (1H, t (5.1 Hz), H-1). ^{13}C NMR δ 27.00 and 34.72 (C-5 and C-4); 47.67 (C-5); 82.35 (C-1); 175.07 (C-3); 216.63 (C-6). Calcd for $C_7H_{12}O_3$: C, 59.99; H, 7.19 %; found: C, 59.91; H, 7.32 %.

(1α,2β,5α)-2-Hydroxybicyclo[3.2.0]heptan-6-one ethylene acetal (rac-***8***). Ketoacetal *rac*-**16** (4.00 g, 23.8 mmol) was dissolved in 100 mL of methanol and cooled to –20 °C. After the addition of 0.38 g (10 mmol) of $NaBH_4$ in small portions the solution was stirred for 1 h until the reaction was finished. After addition of 100 mL of sat. NH_4Cl most of the methanol was removed under vacuum and the remaining milky liquid was extracted 4 times with diethyl ether. The organic phase was washed with sat. $NaHCO_3$ and dried over Na_2SO_4. After concentration under reduced pressure and drying *in vacuo* with an oil pump, 3.93 g (23.1 mmol, 97.0 %) of compound **8** was isolated, which contained no *exo*-isomer **5** according to gas chromatography (column C). ^{1}H NMR δ 1.38–1.58 (1H, m); 1.70–1.95 (3H, m); 2.20 (1H, ddd (14.9 Hz/9.0 Hz/3.0 Hz)); 2.31 (1H, dd (14.0 Hz/7.8 Hz)); 2.36–2.55 (2H, m); 2.71 (1H, dt (2.8 Hz/7.7 Hz); 3.70–3.93 (4H, m, O-CH_2-CH_2-O); 4.19 (1H, dt (9.4 Hz/6.7 Hz)). ^{13}C NMR δ 22.83; 32.27; 32.27; 32.79; 33.08; 50.03; 63.39/64.46 (OCH_2CH_2O); 75.00 (C-2); 107.10 (C-6). Calcd for $C_9H_{14}O_3$: C, 63.51; H, 8.29 %; found: C, 63.57; H, 8.46 %.

(1α,2α,5α)-2-Bromobicyclo[3.2.0]heptan-6-one ethylene acetal (rac-***6***). Alcohol **5** (5.00 g, 29.4 mmol) was brominated according to procedure 4. After purification by chromatography (180 g of silica gel, eluent A) and drying *in vacuo* with an oil pump, 5.25 g (22.5 mmol, 76.6 %) of *endo*-bromoacetal **6** were obtained as a slightly yellow oil. ^{1}H NMR δ 1.38–1.55 (1H, m); 1.79 (1H, dd (12.9 Hz/5.7 Hz)); 2.05–2.42 (4H, m); 2.62–2.78 (2H, m); 3.70–3.95 (4H, m, O-CH_2-CH_2-O); 4.14 (1H, dt (10.7 Hz/6.6 Hz), H-2-*exo*). ^{13}C NMR δ 25.51; 35.01; 35.28; 36.82 (C-5); 53.27 (C-2); 63.49/64.49 (OCH_2CH_2O); 105.35 (C-6). Calcd for $C_9H_{13}BrO_2$: C, 46.37; H, 5.62; Br, 34.28 %; found: C, 46.56; H, 5.81; Br, 34.07 %.

(1α,2α,5α)-2-Bromobicyclo[3.2.0]heptan-6-one (rac-***7***). Acetal **6** (5.19 g, 22.3 mmol) was reacted according to procedure 5. After chromatography (100 g of silica gel, eluent B) and drying *in vacuo* with an oil pump 3.44 g (18.2 mmol, 81.6 %) of a colorless oil was isolated. Bp (KR) 110–120 °C (2 kPa). ^{1}H NMR δ 1.58–1.74 (1H, m); 1.85–2.03 (2H, m); 2.27; (1H, dt (12.7 Hz/7.0 Hz)); 2.94–3.15 (3H, m); 3.41–3.53 (1H, m); 4.28–4.41 (1H, m, H-2-*exo*). ^{13}C NMR δ 28.07 (C-4); 34.63 (C-3); 49.59 (C-1); 51.71 (C-7); 56.80 (C-2); 62.68 (C-5); 210.42 (C-6). Calcd for C_7H_9BrO: C, 44.47; H, 4.80; Br, 42.27 %; found: C, 44.81; H, 4.98; Br, 42.13 %.

(1α,5α,6α)-6-Bromo-2-oxabicyclo[3.3.0]octan-3-one (rac-***7a***). Bromoketone **7** (1.00 g, 5.29 mmol) was oxidized according to procedure 3. Chromatography (30 g of silica gel, eluent B) gave after drying with an oil pump 0.93 g (4.53 mmol, 85.7 %) of bromolactone *rac*-**7a** as colorless oil. According to GC (column D) the compound was contaminated with 5.1 % of the isomeric lactone *rac*-**7b** which could not be removed by liquid chromatography. ^{1}H NMR δ 1.87–2.23 (4H, m); 2.52–2.82 (2H, m); 2.93–3.15 (1H, m, H-5); 4.22–4.40 (1H, m, H-6-*exo*); 4.82–4.97 (1H, m, H-1). ^{13}C NMR δ 31.73; 33.70; 34.91; 43.56; 53.13 (C-6); 83.81 (C-1); 176.54 (C-3).

(1β,2α,5β)-2-Bromobicyclo[3.2.0]heptan-6-one ethylene acetal (rac-***9***). Alcohol **8** (4.20 g, 24.6 mmol) was reacted according to procedure 4. Chromatography (160 g of silica gel, eluent A) gave after drying *in vacuo* with an oil pump 4.85 g (20.8 mmol, 84.6 %) of *exo*-bromide **9**. ^{1}H NMR δ 1.75 (1H, dd (14.0 Hz/6.0 Hz)); 1.84 (1H, dd (7.2 Hz/13.1 Hz)); 1.90–2.16 (2H, m); 2.27–2.39 (1H, m); 2.43 (1H, ddd (3.3 Hz/9.7 Hz/13.7 Hz)); 2.87 (1H, dt (9.3 Hz/6.5 Hz)); 3.98 (1H, dt (3.2 Hz/7.9 Hz)); 3.73–3.88 (4H, m, O-CH_2-CH_2-O); 4.31 (1H, d (4.3 Hz), H-2-*endo*). ^{13}C NMR δ 24.21 (C-4); 35.78 (C-3); 40.03 and 40.52 (C-1 and C-7); 50.89 (C-5); 58.13 (C-2); 63.46 and 64.59

(OCH_2CH_2O); 105.90 (C-6). Calcd for $C_9H_{13}BrO_2$: C, 46.37; H, 5.62; Br, 34.28 %; found: C, 46.52; H, 5.80; Br, 34.37 %.

(1β,2α,5β)-2-Bromobicyclo[3.2.0]heptan-6-one (rac-**10**). Bromoacetal **9** (4.70 g, 20.2 mmol) was reacted according to procedure 5. After chromatography (50 g of silica gel, eluent A) and drying *in vacuo* with an oil pump 3.23 g (17.1 mmol, 84.6 %) of bromoketone **10** was isolated as a colorless oil. Bp (KR) 110–120 °C (2 kPa). ^{1}H NMR δ 1.95–2.33 (4H, m, 2 × H-3, 2 × H-4); 2.48 (1H, ddt (19 Hz/5 Hz/4 Hz)); 3.18 (1H, ddd (19 Hz/10 Hz/5 Hz)); 3.24–3.37 (1H m); 3.65–3.78 (1H, m); 4.50 (1H, d (4 Hz), H-2-*endo*). ^{13}C NMR δ 27.20 (C-4); 35.09 (C-3); 40.00 (C-1); 52.01 (C-7); 56.50 (C-2); 64.48 (C-5); 210.07 (C-6). Calcd for C_7H_9BrO: C, 44.47; H, 4.79; Br, 42.27 %; found: C, 44.87; H, 4.76; Br, 42.16 %.

(1β,5β,6α)-6-Bromo-2-oxabicyclo[3.3.0]octan-3-one (rac-**10a**). Bromoketone *rac*-**10** (300 mg, 1.59 mmol) was oxidized with *m*-CPBA according to procedure 3. After chromatography (10 g of silica gel, eluent B) and drying *in vacuo* with an oil pump 270 mg (1.32 mmol, 83 %) of bromolactone *rac*-**10a** was isolated as a colorless oil. According to GC (column D) it was contaminated with 3.1 % of the isomeric lactone *rac*-**10b** which could not be removed by liquid chromatography. ^{1}H NMR δ 1.95–2.43 (5H, m); 2.83 (1H, dd (11.2 Hz/18.9 Hz), H-4-*exo*); 3.22–3.36 (1H, m, H-5); 4.13–4.23 (1H, m, H-6-*endo*); 5.10 (1H, t (6.3 Hz); H-1). ^{13}C NMR δ 31.47; 34.41 (2 × C); 50.10; 55.08; 84.71 (C-1); 175.75 (C-3).

(1β,2α,5β)-2-Benzyloxybicyclo[3.2.0]heptan-6-one ethylene acetal (rac-**12**). Alcohol *rac*-**5** (3 g, 17.6 mmol) was benzylated according to procedure 6. After chromatography (70 g of silica gel, eluent B) and drying *in vacuo* with an oil pump, 3.84 g (14.7 mmol, 83.7 %) of a colorless oil was isolated. ^{1}H NMR δ 1.65–2.07 (5H, m); 2.40–2.56 (1H, m); 2.56–2.70 (1H, m); 2.87–3.01 (1H, m); 3.70–3.95 (5H, m, H-2-*endo*, O-CH_2-CH_2-O); 3.46 (2H, s, OCH_2Ph), 7.18–7.39 (5H, m, C_6H_5). ^{13}C NMR δ 24.58; 30.86; 35.05; 37.51; 50.93; 63.41 and 64.62 (O-CH_2-CH_2-O); 70.41 (C-2); 85.39 (O-CH_2Ph); 107.25 (C-6); 127.56/127.68 (2 × C)/128.53 (2 × C)/138.73 (all C_6H_5). Calcd for $C_{16}H_{20}O_3$: C, 73.82; H, 7.74 %; found: C, 74.17; H, 7.68 %.

(1β,2α,5β)-2-Benzyloxybicyclo[3.2.0]heptan-6-one (rac-**13**).[21] Acetal *rac*-**12** (3.50 g, 13.4 mmol) was dissolved in 200 mL of acetone and stirred with 1.5 mL of conc. H_2SO_4 until no more starting material (3 h) could be detected by TLC (eluent B, VS). Then, 100 mL of saturated $NaHCO_3$ was added and the mixture was stirred for another hour. After removal of the acetone the aqueous residue was mixed with H_2O and extracted four times with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and purified by chromatography (60 g of silica gel, eluent B). After drying *in vacuo* with an oil pump 2.64 g (12.2 mmol, 91.0 %) of ketone *rac*-**13** was isolated as a colorless oil. ^{1}H NMR δ 1.60–1.76 (1H, m); 1.87–2.16 (3H, m); 2.40 (1H, ddd (18.4 Hz/4.3 Hz/3.4 Hz), H-7-*endo*); 2.88–3.03 (1H, m, H-1); 3.16 (1H, ddd (18.2 Hz/9.6 Hz/4.7 Hz), H-7-*exo*); 3.55–3.68 (1H, m, H-5); 3.97 (1H, d (3.9 Hz), H-2-*endo*); 4.54 (2H, s, OCH_2Ph); 7.25–7.38 (m, 5H, C_6H_5). ^{13}C NMR δ 27.13 (C-4); 30.04 (C-3); 34.57 (C-1); 49.35 (C-7); 64.32 (C-5); 70.22 (OCH_2Ph); 84.55 (C-2); 127.39 (2 × C)/127.53/128.31 (2 × C)/138.54 (all C_6H_5); 212.59 (C-6). Calcd for $C_{14}H_{16}O_2$: C, 77.75; H, 7.46 %; found: C, 77.35; H, 7.68 %.

(1β,5β,6α)-6-Benzyloxy-2-oxabicyclo[3.3.0]octan-3-one (rac-**13a**). Ketone *rac*-**13** (840 mg, 3.88 mmol) was reacted according to procedure 3. Chromatography (30 g of silica gel, eluent B) and drying *in vacuo* with an oil pump gave 620 mg (2.67 mmol, 68.8 %) of lactone *rac*-**13a** as a colorless oil. The product contained 8 % of the isomer lactone *rac*-**13b** (GC, column C). ^{1}H NMR δ 1.69–1.83 (1H, m); 1.92–2.24 (3H, m); 2.29 (1H, dd (17.8 Hz/2.8 Hz), H-4-*endo*); 2.85 (1H, dd (17.8 Hz/11.6 Hz), H-4-*exo*); 2.88–3.00 (1H, m, H-5); 3.77–3.83 (1H, m, H-6); 4.48 (2H, AB (Δ = 0.05 ppm/J = 11.8 Hz), OCH_2Ph); 5.10 (1H, t (5.8 Hz), H-1); 7.25–7.42 (5H, m, $OCH_2C_6H_5$). ^{13}C NMR δ 28.79; 31.04; 33.40; 45.05; 70.94 (OCH_2Ph); 85.42 and 86.32 (C-1 and C-6); 127.73 (2 × C)/127.93/128.67 (2 × C)/138.34 (all C_6H_5); 176.96 (C-3).

(1β,2α,5β)-2-Benzyloxybicyclo[3.2.0]heptane-6-one ethylene acetal (rac-**14**). Alcohol *rac*-**8** (3.39 g, 19.9 mmol) was benzylated according to procedure 6. After chromatography (80 g of silica gel, eluent B) and drying *in vacuo* with an oil pump 4.48 g (17.2 mmol, 86.5 %) of a colorless oil was isolated. ^{1}H NMR δ 1.48 (1H, tt (11.5 Hz/8 Hz)); 177–2.05 (3H, m); 2.27 (1H, ddd (13.7 Hz/9 Hz/3.4 Hz)); 2.46 (1H, dd (14.0 Hz/5.9 Hz)); 2.62 (1H, dq (9.1 Hz/6.6 Hz); 2.79 (1H, dt (9.0 Hz/2.7 Hz)); 3.77–4.06 (5H, m, H-2-*exo*, O-CH_2-CH_2.O); 4.46 (2H, s, OCH_2Ph); 7.23–7.45 (5H, m, C_6H_5). ^{13}C NMR δ 22.63 (C-4); 29.49; 30.71; 33.10; 49.66 (C-5); 63.29 and 64.46 (O-CH_2-CH_2-O); 71.71 (O-CH_2Ph); 81.71 (C-2); 107.09 (C-6); 127.59/127.77/128.41/138.96 (all arom.). Calcd for $C_{16}H_{20}O_3$: C, 73.82; H, 7.74 %; found: C, 74.54; H, 7.73 %.

(1α,2α,5β)-2-Benzyloxybicyclo[3.2.0]heptan-6-one (rac-**15**). Acetal *rac*-**14** (4.48 g, 17.2 mmol) was dissolved in 400 mL of acetone and stirred with 0.5 mL of conc. H_2SO_4 until no more starting material (3 h) could be detected by GC (column C). Then 100 mL of sat. $NaHCO_3$ was added and the mixture was stirred for another hour. After removal of the acetone, water was added and extracted four times with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and purified by chromatography (120 g of silica gel, eluent B). After drying *in vacuo* with an oil pump 3.56 g (16.4 mmol, 95.3 %) of ketone *rac*-**15** was isolated as a colorless oil. ^{1}H NMR δ 1.54–1.78 (2H, m); 1.87–198 (1H, m); 2.01–2.15 (1H, m); 2.85–3.04 (2H, m); 3.12 (1H, ddd (18.6 Hz/8.1 Hz/3.0 Hz), H-7-*exo*); 3.41–3.53 (1H, m); 4.17 (1H, dt (10.0 Hz/6.3 Hz), H-2-*exo*); 5.52 (2H, s, OCH_2Ph); 7.23–7.45 (5H, m C_6H_5). Calcd

for $C_{14}H_{14}O_2$: C, 77.75; H, 7.46 %; found: C, 78.20; H, 7.41 %.

(1α,5α,6β)-6-Benzyloxy-2-oxabicyclo[3.2.0]heptan-6-one (rac-***15a***). Ketone *rac*-**15** (0.30 g, 1.39 mmol) was oxidized with *m*-CPBA according to procedure 3. Chromatographic purification (10 g of silica gel, eluent B) and drying *in vacuo* with an oil pump gave 262 mg (1.12 mmol, 80 %) of lactone *rac*-**15a** which, according to GC (column C), was contaminated with 6.6 % of the isomeric lactone *rac*-**15b**.

(1β,2α,5β)-2-Hydroxybicyclo[3.2.0]heptan-6-one (rac-***17***). Hydroxyacetal *rac*-**5** (3.10 g, 18.2 mmol) was deprotected according to procedure 5. Because of the good solubility of the product in diluted aqueous acetic acid, workup was carried out by perforation with CH_2Cl_2. After chromatography (80 g of silica gel) and Kugelrohr distillation 1.93 g (15.3 mmol, 84 %) of a viscous oil were obtained. Bp (KR) 140–150 °C (2 kPa). ^{1}H NMR δ 1.63–2.02 (4H, m); 2.37 (1H, ddd (18.8 Hz/4.8 Hz/3.5 Hz), H-7-*endo*); 2.70–2.93 (2H, m, OH, H-1); 3.09 (1H, ddd (18.8 Hz/9.8 Hz/4.7 Hz), H-7-*exo*); 3.53–3.64 (1H, m, H-5); 4.22 (1H,d (3.5 Hz), H-2-*endo*). ^{13}C NMR δ 26.90 (C-4); 33.15 (C-3); 37.47 (C-1); 49.20 (C-7); 64.20 (C-5); 77.23 (C-2); 213.67 (C=0). Calcd for $C_7H_{10}O_2$: C, 66.65; H, 7.99 %; found: C, 66.49; H, 8.04 %.

(1α,5α,6β)-6-Hydroxy-2-oxabicyclo[3.3.0]octan-3-one (rac-***17a***). Hydroxyketone *rac*-**17** (126 mg, 1.00 mmol) was reacted according to procedure 3. After chromatography (8 g of silica gel, eluent D) and drying *in vacuo* with an oil pump 52 mg (0.37 mmol, 37 %) of hydroxylactone *rac*-**17a** were isolated as a colorless oil. The ^{1}H NMR spectrum showed a 4 % contamination with the isomeric lactone *rac*-**17b** which could not be removed by liquid chromatography. ^{1}H NMR δ 1.63–1.90 (2H, m); 1.90–2.06 (1H, m); 2.06–2.21 (1H, m); 2.28 (1H, dt (14.7 Hz/9.7 Hz), H-4-*endo*); 3.56 (1H, brs, H-5); 2.81 (1H, dt (14.7 Hz/11.2 Hz); H-4-*exo*); 4.08 (1H, brs, H-6-*endo*); 5-10 (1H, brt (5.5 Hz), H-1). ^{13}C NMR δ 30.61; 33.16; 47.36 (C-5); 78.90 (C-6); 85.81 (C-1); 177.44 (C-3).

cis-*2-Oxabicyclo[3.3.0]octan-3-one* (rac-***11a***). Unsaturated lactone *rac*-**18a**[19] (0.66 g, 5.2 mmol) was hydrogenated in ethanol with 100 mg of Pd/C 10 % under atmospheric pressure. After removal of the catalyst by filtration through celite and careful evaporation of the ethanolic filtrate at 40 °C water bath temperature, chromatography (8 g of silica gel, eluent B) gave 200 mg of pure, saturated lactone *rac*-**11a**. ^{1}H NMR δ 1.45–1.90 (5H, m); 1.98–2.08 (1H, m); 2.28 (1H, d (15.7 Hz), H-4-*endo*); 2.82 (1H, dd (15.6 Hz/10.2 Hz), H-4-*exo*); 2.80–2.93 (1H, m, H-5); 4.99 (1H, t (5.4 Hz), H-1). ^{13}C NMR: 23.63; 33.68; 33.82; 36.25; 38.21; 86.61 (C-1); 177.94 (C-3). Calcd for $C_7H_{10}O_2$: C, 66.65; H, 7.99 %; found: C, 66.35; H, 7.96 %.

*(1R, 5S)-3-Oxabicyclo[3.3.0]octan-2-one (**11b**).* Lactone **18b** (160 mg, 1.3 mmol) obtained from the microbial Baeyer–Villiger reaction of ketone *rac*-**18**[12] was hydrogenated in 10 mL of ethanol with 50 mg of Pd/C 5 %. Filtration after 15 h through Celite, evaporation of the solvent *in vacuo* and drying with an oil pump gave 133 mg (82 % yield) of lactone **11b**, after purification by preparative GC a colorless oil, $[\alpha]_D^{20}$ –95.9 (*c* 3.19; $CHCl_3$), lit.[15] for the (1*S*, 5*R*)-enantiomer $[\alpha]_D^{20}$ + 96.9 (*c* 1; $CHCl_3$).

Biotransformations using Acinetobacter calcoaceticus *NCIB 9871.* The following nutrient medium was used: component A: 2.0 g KH_2PO_4, 4.0 g Na_2HPO_4, 3.0 g $(NH_4)_2SO_4$, 0.2 g yeast extract, 970 mL distilled water; component B: 50.0 g/L $MgSO_4 \cdot 7H_2O$; component C: 10.0 g/L $CaCl_2 \cdot H_2O$; component D: 1.0 g/L $FeSO_4 \cdot 7H_2O$. These components were sterilized separately and after cooling to room temperature 10 mL of each component B, C and D were added to component A. For the preparative fermentations a 2 L fermenter from Setric Genie Industriel, type SET 2 was used. For a typical fermentation, 1070 mL of nutrient medium component A, sterilized for 30 min at 121 °C, was used. After cooling to 30 °C the nutrient medium in the bioreactor was completed by addition of 11.0 mL each of components B, C and D. In order to serve as a carbon source for the growth of the microorganisms 1.65 mL of cyclohexanol were added. Subsequently the fermentation was started by inoculating the bioreactor with 20 mL of a stage 1 culture grown for 24 h in an Erlenmeyer flask (300 mL, 4 baffles). Fermentation was performed at 30 °C, at an agitator speed of 500 rpm, and an aeration rate of 20 L/h. During the growth phase of the experiment the dissolved oxygen concentration decreased to nearby 0 mg/L. In the moment when the cyclohexanol in the medium was depleted the oxygen concentration in the medium rose sharply to about 90 % of the saturation value for air. At this point temperature was reduced to 25 °C and 200 µL of tetraethyl pyrophosphate were added as an esterase inhibitor. Forty-five minutes later 550 mg of *trans*-cyclohexane-1,2-diol dissolved in 5 mL of water was added. The dissolved oxygen concentration started to drop again and when it was nearly zero, 800–1000 mg of starting material, dissolved in 5 mL of ethanol was added. The culture was kept under oxygen limiting conditions and the fermentation was monitored by chromatography of samples drawn. After the end of the fermentation the fermentation broth was extracted for 48 h with CH_2Cl_2. The organic phase was dried over Na_2SO_4, concentrated *in vacuo*, and the products were purified by column chromatography as described for the racemic compounds.

Biotransformation of rac-*7*. Starting from 1.0 g (5.29 mmol) of *rac*-**7** after 220 min of fermentation, workup as described above and chromatography (50 g of silica gel, eluent D) 1.01 g (93 % yield) of 66 % **7a** and 34 % **7b** was obtained (GC, column C) which could not be separated by preparative liquid chromatography. The enantiomeric excess was determined by GC (column D). The assignment of absolute configuration was performed by debrominating the mixture of lactones **7a/7b** (200 mg, 0.97 mmol) according to procedure 2. After chromatography (15 g of silica gel, eluent C) 110 mg (0.87 mmol, 90 %) of a mixture of (1*S*,5*S*)-**11a** and (1*R*,5*S*)-**11b** was obtained, the absolute configuration of which was established by comparison (GC, column D) with (1*R*,5*R*)-**11a** obtained by catalytic hydrogenation of (1*S*,5*R*)-**18a**[13] and

enantiomerically enriched (1*R*,5*S*) **11b** from the biotransformation of *rac*-**18** followed by hydrogenation.

(1S,5S,6S)-6-Bromo-3-oxabicyclo[3.3.0]octan-2-one (7b). This compound could only be obtained as a mixture with **7a**. The ^{1}H NMR spectrum was not interpreted due to overlap of the signals. ^{13}C NMR δ 27.78 and 36.39 (C-7 and C-8); 43.08 and 44.40 (C-1 and C-5); 54.2 (C-6); 70.25 (C-4); 179.5 (C-2).

Biotransformation of rac-*10*. Analogously *rac*-**10** gave after complete conversion (120 min), extraction with methyl *tert*butyl ether and chromatography 860 mg (79 % yield) of a mixture of 62 % **10a** and 38 % **10b** which could not be separated by preparative liquid chromatography. The procedure for the determination of the enantiomeric excess and the absolute configuration was the same as described for **7a/7b**.

(1R,5S,6R) -6- Bromo -3- oxabicyclo [3.3.0] octan -2- one (10b). This compound could only be obtained as a mixture with **10a**. ^{1}H NMR δ 1.93–2.46 (4H, m); 3.11 (1H, dt (2.0 Hz/9.6 Hz); 3.20–3.33 (1H, m, H-5); 3.96 (1H, dd (3.6 Hz/11.2 Hz), H-4-*endo*); 4.12–4.20 (1H, m, H-6); 4.41 (1H, dd (8.6 Hz/11.2 Hz), H-4-*exo*). ^{13}C NMR δ 28.58 and 36.25 (C-7 and C-8); 43.18 and 50.71 (C-1 and C-5); 5.57 (C-6); 71.30 (C-4); 179.50 (C-2).

Biotransformation of rac-*13*. Analogously to the transformation of *rac*-**10** but in a much slower reaction (18.5 h for 98 % conversion) after chromatography (eluent B) 760 mg (71 % yield) of a mixture of 54 % **13a** and 46 % **13b** was obtained which could not be separated by preparative liquid chromatography. For the determination of the enantiomeric excess 70 mg of this mixture was hydrogenolyzed in 5 mL of ethanol using 30 mg Pd/C 10 % as catalyst. The alcohols formed (**17a/17b**) were then brominated according to procedure 4 to give bromides **7a/7b** the enantiomeric composition and the absolute configuration of which was determined by GC (column D) as described above.

(1R,5S,6R)-6-Benzyloxy-3-oxabicyclo 3.3.0.] octan -2- one (13b). From the mixture with **13a** the NMR data were determined. ^{1}H NMR δ (most important signals): 3.11 (1H, dt (1.5 Hz/9.4 Hz), H-1); 3.98 (1H, dd (1H, dd 9.6 Hz/3.7 Hz), H-4-*exo*). ^{13}C NMR δ 28.16 and 30.72 (C-7 and C-8); 43.08 (C-5); 45.92 (C-1); 70.80 and 70.86 (C-4 and OCH_2Ph); 86.26 (C-6); 127.63 (2 × C)/127.84/128.87 (2 × C)/138.13 (all C_6H_5); 180 (C-2).

Biotransformation of rac-*15*. Analogously to the transformation of *rac*-**10**, after 4.5 h complete conversion was achieved to give, after chromatography (eluent B), 850 mg (79 % yield) of a mixture of 32 % *rac*-**15a** and 68 % **15b** which could not be separated by preparative column chromatography. For the determination of the enantiomeric excess and the absolute configuration the procedure was analogous to that described for **13a/13b**.

(1R,5S,6S)-6-Benzyloxy-3-oxabicyclo [3.3.0] octan -3- one (15b). From the mixture with **15a** the NMR data were determined. ^{1}H NMR δ (most important signals): 4.02 (1H, m, H-6); 4.23 (m, 1H) and 4.44 (1H, m), (2 × H-4); 4.59 (2H, m, OCH_2Ph). ^{13}C NMR δ 26.50 and 30.40 (C-7 and C-8); 41.75 and 43.01 (C-1 and C-5); 66.92 (C-4); 71.73 (OCH_2Ph); 81.12 (C-6); 127.54 (2 × C)/128.06/128.58 (2 × C)/134.24 (all C_6H_5); 180.79 (C-2).

Biotrdnsformation of rac-*17*. Analogously to the transformation of *rac*-**7**, after 4.5 h complete conversion was achieved to give after chromatography 849 mg (75 % yield) of a mixture of 52 % **17a** and 48 % **17b** which could not be separated by preparative chromatography. The procedure for the determination of the enantiomeric excess and the absolute configuration was analogous to that described for **13a/13b**.

(1R,5S,6R)-6-Hydroxy-3-oxabicyclo [3.3.0] octan -2- one (17b). ^{1}H NMR δ (most important signals): 3.07 (1H, dt (2.2 Hz/9.4 Hz)); 3.97 (1H, dd (9.7 Hz/3.7 Hz), H-4-*endo*); 4.07 (1H, dt (4.2 Hz/2.6 Hz), H-6-*endo*); 4.42 (1H, dd (9.7 Hz/9.4 Hz), H-4-*exo*). ^{13}C NMR δ 27.84 and 33.95 (C-7 and C-8); 42.84 and 48.06 (C-1 and C-5); 70.96 (C-4); 78.75 (C-6); 181.38 (C-2).

Biotransformation of rac-*18*. Compound *rac*-**18** (900 mg) gave analogously to the transformation of *rac*-**7** after 5.5 h (complete conversion) and chromatography 370 mg (36 % yield) of **18a** (96 % *e.e*, column D) and 340 mg (33 % yield) of **18b** (> 99 % *e.e.* column D).

(1R,5S)-2-Oxabicyclo[3.3.0]oct-7-en-3-one (18a). Colorless oil, $[\alpha]_D^{20}$ + 136.6 (*c* 3.84; $CHCl_3$); lit. [22] for (1*S*,5*R*)-**18a** (about 80 % *e.e.*) $[\alpha]_D^{20}$ –98 (*c* 4.2, CH_2Cl_2). ^{1}H NMR δ 2.19 and 2.21 (2H, m, 2 × H6); 2.66 (1H, m, H-4a); 2.74 (1H, dd (18.4 Hz/10.4 Hz) H-4b); 3.05 (1H, m, H-5); 5.41 (1H, m, H-1); 5.75 (1H, ddd (2.1 Hz/4.3 Hz/5.7 Hz) H-7); 5.98 (1H, m H-8). ^{13}C NMR δ 34.83 (C-5), 35.74 and 39.34 (C-4, C-6); 87.37 (C-1); 128.67 (C-7) 136.88 (C-8); 176.88 (C-3).

(1R,5S)-3-Oxabicyclo[3.3.0]oct-7-en-2-one (18b). For the determination of the absolute configuration **18b** was hydrogenated (5 % Pd/C, ethanol) to give **11b** (see above). Colorless oil, $[\alpha]_D^{20}$ –331 (*c* 3.9, $CHCl_3$). ^{1}H NMR δ 2.26 (1H, dm (15.9 Hz), H-6-*endo*); 2.67 (1H, ddm (16.5 Hz/8.0 Hz), H-6-*exo*); 3.14–3.28 (1H, m, H-5); 3.52–3.63 (1H, m, H-1); 3.80 (1H, ddd (8.2 Hz/6.5 Hz/1.2 Hz), H-4-*exo*); 4.51 (1H, dt (1.2 Hz/8.3 Hz), H-4-*endo*); 5.62–5.72 (1H, m) and 5.76–5.88 (1H, m), (H-7 and H-8). ^{13}C NMR δ 37.06/39.10 (C-5, C-6); (C-1); 74.29 (C.4); 126.17 (C-7); 132.81 (C-8); 176.94 (C-2). Calcd for $C_7H_8O_2$: C, 67.37; H, 6.50 %; found: C, 68.29; H, 6.37 %.

Biotransformation of rac-*1*. Compound *rac*-**1** (1.0 g 8.05 mmol) was reacted analogously to the procedure described for *rac*-**7** and gave after 145 min (complete conversion) and chromatography (eluent B, then C, D and E), 320 mg (28 %) of **1a**, 86 mg (7 %) of **1b**, 145 mg (12 %) of a mixture of 53 % of **1d** and 47 % of **1e** and 147 mg (16 %) of **1f**.

(1S,5S)-2-Oxabicyclo[3.3.0]octan-3,6-dione (1a). Colorless oil, $[\alpha]_D^{20}$ +142 (*c* 4.15, $CHCl_3$) (92 % *e.e.*). Calcd for $C_7H_{12}O_3$: C, 59.99; H, 7.19 %; found: C, 59.01; H, 7.42 %.

*(1S,5R,6R)-6- Hydroxy -3- oxabicyclo [3.3.0] octan -2- one (**1b**).* This compound could not be separated by preparative liquid chromatography from **1a**. The enantiomeric excess and the absolute configuration were obtained by transformation into the corresponding bromides and comparison by GC as it is described for **7a**.

*(1α,5α,6α) -6-Hydroxy -2- oxabicyclo[3.3.0] octan -2- one (*rac*-**1d**).* By reduction of 41 mg (0.29 mmol) of ketone **1a** in 1.5 mL of methanol at –20 °C using 11 mg (0.29 mmol) of $NaBH_4$ (30 min stirring), workup by addition of 3 mL of saturated aqueous NaCl, removal of methanol *in vacuo*, acidification with 1 N HCl, extraction with CH_2Cl_2, drying (Na_2SO_4) and chromatography on 3 g of silica gel 23 mg of *rac*-**1d** was obtained. ^1H NMR δ 1.65–3.13 (4H, m); 2.53 (1H, dd (19.0 Hz/11.0 Hz); H-4-*endo*); 2.80–2.89 (1H, m, H-5); 2.87 (1H, dd (19.0 Hz/2.8 Hz), H-4-*exo*); 4.27 (1H, q (6.0 Hz), H-6-*exo*); 4.96 (1H, dt (2.1 Hz/6.5 Hz), H-1). ^{13}C NMR δ 28.59; 29.95; 32.34; 42.73 (C-5); 72.69 (C-6); 84.92 (C-1); 178.24 (C-3).

*(1R,5S,6S)-6-Hydroxy-3-oxabicyclo [3.3.0]octan-2-one (**1e**).* Since this compound could not be separated from **1d** by preparative chromatography the enantiomeric excess and the absolute configuration has been determined after bromination as above (see Table 1).

*(1α,5α,6α)-6-Hydroxy -3- oxabicyclo [3.3.0] octan-2-one (*rac*-**1e**).* By reduction of **1b** using $NaBH_4$ analogously to the reduction of **1a** described above again a racemic product was obtained. ^1H NMR δ 1.59–1.76 (1H, m); 1.80–1.95 (1H, m); 1.95–2.15 (2H, m); 2.48 (1H, d (3.3Hz), OH); 2.87–2.99 (1H, m, H-5); 3.04 (1H, dt (3.6 Hz/9.5 Hz); 4.25–4.40 (2H, m, H-4-*exo*, H-6); 4-59 (1H, dd (9.6 Hz/3.6 Hz), H-4-*endo*). ^{13}C NMR δ 26.45 and 34.55 (C-7 and C-8); 43.27 and 43.79 (C-1 and C-5); 67.12 (C-4); 74.07 (C-6); 181.53 (C-2).

(1R,6S*)-3,8-Dioxabicyclo[4.3.0]nonan-2,7-dione (**1f***).* Absolute configuration and enantiomeric excess have not been determined. Colorless crystals, mp 94–102 °C; $[\alpha]_D^{20}$ –112 (*c* 2.55; $CHCl_3$). ^1H NMR δ 1.91–2.07 (1H, m, H-5-*endo*); 2.26–2.38 (1H, m, H-5-*exo*); 3.29 (1H, dt (11.0 Hz/8.2 Hz), H-4-*endo*); 3.47 (1H, ddd (11.0 Hz/9.0 Hz/3.0 Hz), H-4-*exo*); 4.24–4.42 (2H, m, H-1, H-6); 4.47 (1H, dd (9.7 Hz/8.3 Hz), H-9-*endo*); 4.85 (1H, dd (9.7 Hz/2.9 Hz), H-9-*exo*). ^{13}C NMR δ 23.51 (C-5); 36.38 and 38.97 (C-1 and C-6); 67.08 and 68.05 (C-4 and C-9); 169.89 (C-2); 176.61 (C-7).

(1R,5S)-3-Oxabicyclo[3.3.0]octan-2,6-dione, cyclosarkomycin (**1b**). (a) By microbial Baeyer–Villiger reaction of *rac*-**1**. As described above **1b** was isolated from the mixture of products of the fermentation of *rac*-**1** by chromatography in 7 % yield. Colorless crystals, mp 59–61 °C; $[\alpha]_D^{20}$ –408 (*c* 2.19; $CHCl_3$, determined from a product obtained according to procedure (b) with 98 % *e.e.*) (lit.[5c] mp 59.5–60 °C; $[\alpha]_D^{20}$ –397 (*c* 200; CH_2Cl_2).

(b) By oxidation of **17a/17b**. Microbial Baeyer–Villiger reaction of *rac*-**17** furnished in 75 % yield a mixture of 52 % **17a** (40 % *e.e.*) and 48 % **17b** (> 98 % *e.e.*) as described above. This mixture (600 mg) was oxidized according to procedure 1. After preparative column chromatography on 30 g of silica gel (eluent hexane : ethyl acetate 2:1) and drying with an oil pump besides 178 mg (30 %) of **1a** 157 mg (26 %) of cyclosarkomycin (**1b**) was obtained.

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References and Notes

1. Taken in part from the PhD thesis of Kurt Königsberger, Graz University of Technology, 1992.

2. In case of racemic compounds only one enantiomer is shown.

3. Neither the ratio of enantiomers formed nor the absolute configuration has been determined for **1f***.

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Note Added in Proof: Recently a synthesis of cyclosarkomycin has been published using Sharpless epoxidation followed by iodocarbocyclization: Kitagawa, O.; Inoue, T.; Taguchi, T. *Tetrahedron Lett.* **1994**, *35*, 1059.