18. High-Yield Synthesis of 20-, 24-, and 28-Membered Macropentolide, -hexolide, and -heptolide, Respectively, from (*R*)- or (*S*)-3-Hydroxybutanoic Acid under *Yamaguchi*'s Macrolactonization Conditions[1])

by **Dieter Seebach***, **Urs Brändli**[2]), and **Peter Schnurrenberger**[3])

Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, ETH-Zentrum, Universitätstrasse 16, CH–8092 Zürich

and **Michael Przybylski**[4])

Institut für Organische Chemie, Johannes-Gutenberg-Universität, J.-J.-Becher-Weg 18–20, D–6500 Mainz

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The macrocyclic pentolide **1**, hexolide **2**, and heptolide **3** constitute *ca.* 80% of the oligomers formed in *ca.* 50% yield from enantiomerically pure 3-hydroxybutanoic acid under *Yamaguchi*'s macrolactonization conditions. The FAB mass spectra of the MH^+, MNa^+, and MCs^+ are reported (*Figs. 2, 3, 5,* and *6*). No cyclic tetramer is detected. The ^{1}H-NMR spectra of the cyclic oligomers, of the monomer, and of the polymer (PHB) are very similar (*Fig. 4*). Directed synthesis of the open-chain dimer and tetramer of 3-hydroxybutanoic acid and attempted cyclization do not lead to the isolation of the cyclic tetramer.

A) Introduction – a Weird Idea. – There is a group of macrodiolides with C_2-symmetry containing 16-membered rings as the central most characteristic structural moiety: pyrenophorine, vermiculine, conglobatine, and elaiophyline (**A** in *Fig. 1*; see review [2] and ref. cit. therein). With the exception of conglobatine [3], these fungal metabolites have antibiotic activity, in some cases even irrespective of their sense of chirality or substitution pattern. Thus, both enantiomers of pyrenophorine (**A**, $R^1 = CH_3$, FG = 5,13-dioxo) and the achiral norpyrenophorine (**A**, R^1 = H, FG = 5,13-dioxo) all exhibit very similar activity towards a number of microorganisms [4]. In the course of our work on syntheses of these compounds [2–6] and as a consequence of our interest in 3-hydroxybutanoic acid as a chiral building block (for reviews, see [1] [7]), it occurred to us that the tetramer **B** of this acid might also be antibiotic[5]). That this is a rather naive conception became immediately evident upon comparison of the X-ray crystal structures of the diolides (for instance **C** [11]) and a tetrolide (**D** [12] [13] in *Fig. 1*). On the other

[1]) For a partial preliminary communication, see our recent review on the use of the biopolymer PHB/PHV for EPC syntheses [1].

[2]) Most results reported in this paper are part of the planned Ph. D. thesis of *U. B.*, ETH Zürich.

[3]) First observation of the selective macrocyclization described here (Zürich, 1984); present address of *P. Sch.*: *F. Hoffmann-La Roche & Co. AG*, Bau 29/205, VP/BS, Grenzacherstrasse 124, CH–4002 Basel.

[4]) Measurement and interpretation of the fast-atom-bombardment (FAB) mass spectra.

[5]) Cyclic oligomers of hydroxy acids are structural components of many interesting natural products, such as enterobactines [8], nonactin [9a], boromycin [9b], see also the monograph by *Dobler* [10].

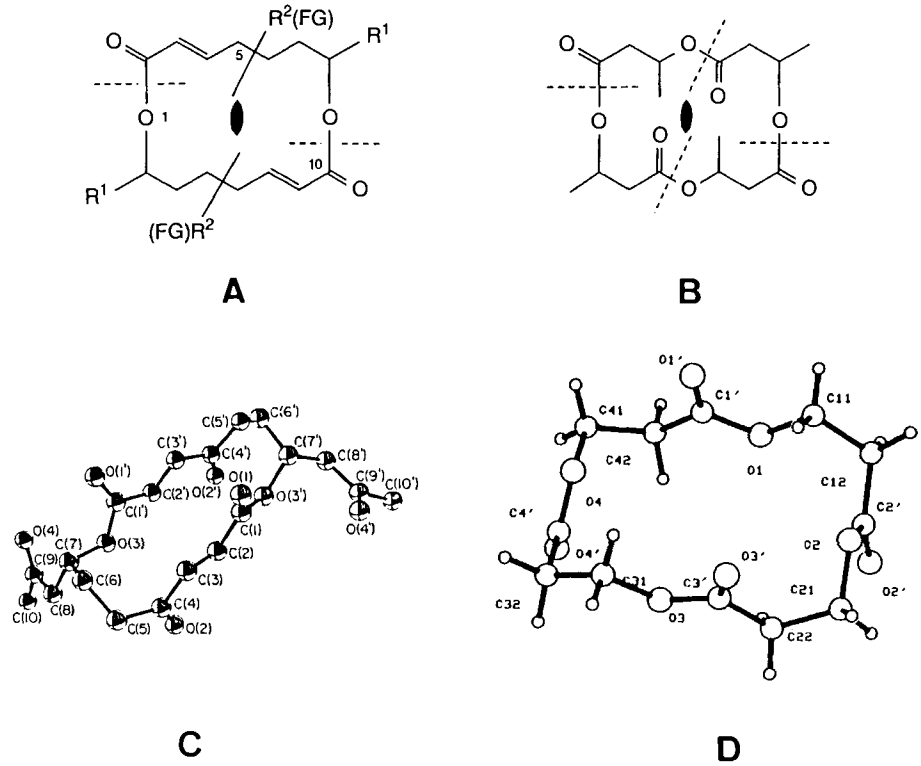


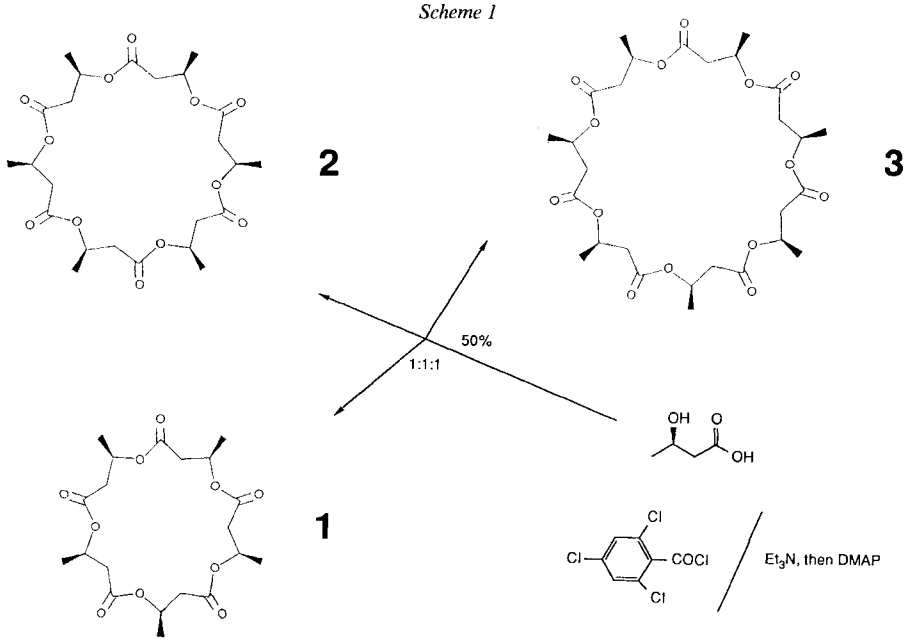
Fig. 1. *Comparison of* C_2*-symmetrical macrodiolide natural products* (**A**,**C**) *with macrotetrolides from 3-hydroxypropanoic* (**D**) *and -butanoic acid* (**B**). **A:** General formula representing the natural products pyrenophorine, vermiculine, elaiophyline, and conglobatine [2] [3]; **B:** 16-membered ring of tetrolide from 3-hydroxybutanoic acid; C_2-symmetrical structure from four acid moieties of (*R*)- or of (*S*)-chirality sense; **C:** X-ray crystal structure of the macrodiolide vermiculine [11]; **D:** X-ray crystal structure of the tetrolide from 3-hydroxypropanoic acid [12] [13].

hand, molecular modelling, using the coordinates[6]) of the X-ray structure **D**, indicated to us that it is quite simple to 'add the methyl groups': no severe steric restrictions were noticed on going from **D** to the tetrolide from (*R*)-3-hydroxybutanoic acid. Thus, we decided to try the synthesis, not foreseeing what endeavor we had embarked for.

B) Macrolactonization of 3-Hydroxybutanoic acid – a Strange Result. – The first experiment led to a very surprizing result: we treated (*R*)-3-hydroxybutanoic acid [14] under *Yamaguchi*'s macrocyclization conditions [15] and isolated three and only three readily separable cyclic oligomers, the pentamer **1**, the hexamer **2**, and the heptamer **3** in approximately equal amounts and in a total yield of *ca.* 50%! As indicated in *Scheme 1*, the method involves formation of a mixed anhydride between a hydroxy acid and 2,4,6-trichlorobenzoic acid at normal concentration (*ca.* 0.5M), and subsequent lactonization by slow addition to 0.0025M 4-(dimethylamino)pyridine (DMAP; reviews: [16]) in

[6]) We thank Professor *A. Shanzer* of the Weizman Institute (Rehovot, Israel) for sending us the hithero unpublished set of coordinates of this crystal structure.

Scheme 1



toluene under high-dilution conditions at room temperature[7]). The crude product obtained in the present case was a yellow oil (*ca.* 85% yield) which was chromatographed on a 2-g scale to give the three crystalline cyclic oligomers **1**–**3** without difficulty (15–20% yield each). The osmometric molecular-weight determinations of the three samples thus isolated gave values of 411, 502, and 654 (calc. 430, 516, and 602, respectively). Several recrystallizations produced the very pure oligomers with sharp melting points (103, 113, and 119°, respectively). The fast-atom-bombardment mass spectra (FAB-MS; *Fig. 2*) and the ^{1}H-NMR spectra *(Fig. 4)* prove the identity of the pentamer, hexamer, and heptamer beyond doubt. As can be seen from *Fig. 3*, the crude product contains a total of *ca.* 80% of the three oligomers **1**–**3** as compared to the higher-molecular weight analogues.

The crude lactonization mixture was analyzed by direct FAB-MS of the protonated components, as well as of the cationized (Na^+ or Cs^+) species. In these latter spectra, there are no signals of molecular ions shifted relative to the MH^+ ions for the tetramer (see arrows in *Figs. 3, 5*, and *6, B* and *C*). The peak of the MH^+ mass for the tetramer (345 in *Fig. 3, A*) is the result of fragmentation. This follows from linked-scan metastable ion spectra (not reported here) the fragmentation pattern of which is interpreted in the *Exper. Part* (see *Scheme 3, Exper. Part*). Thus, the FAB-MS investigations prove that no more than a trace of the cyclic tetramer has been formed.

The octamer which constitutes 10–15% of the mixture was searched for in the chromatography fractions leaving the column after the heptamer – without success. As we noticed that the osmometrically determined molecular weight of the heptamer deviates towards higher values by almost 10%, we suspected that it may be contaminated

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[7]) This method had served us best in most of our macrodiolide syntheses for direct cyclisation of the precursor hydroxy acids [2–6].

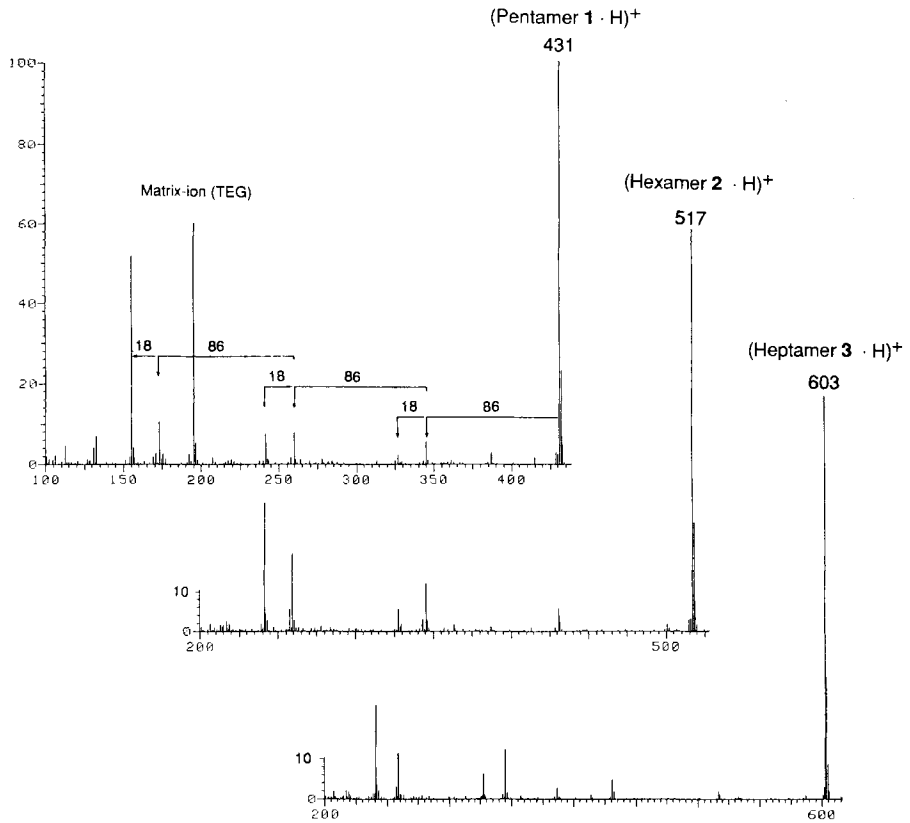
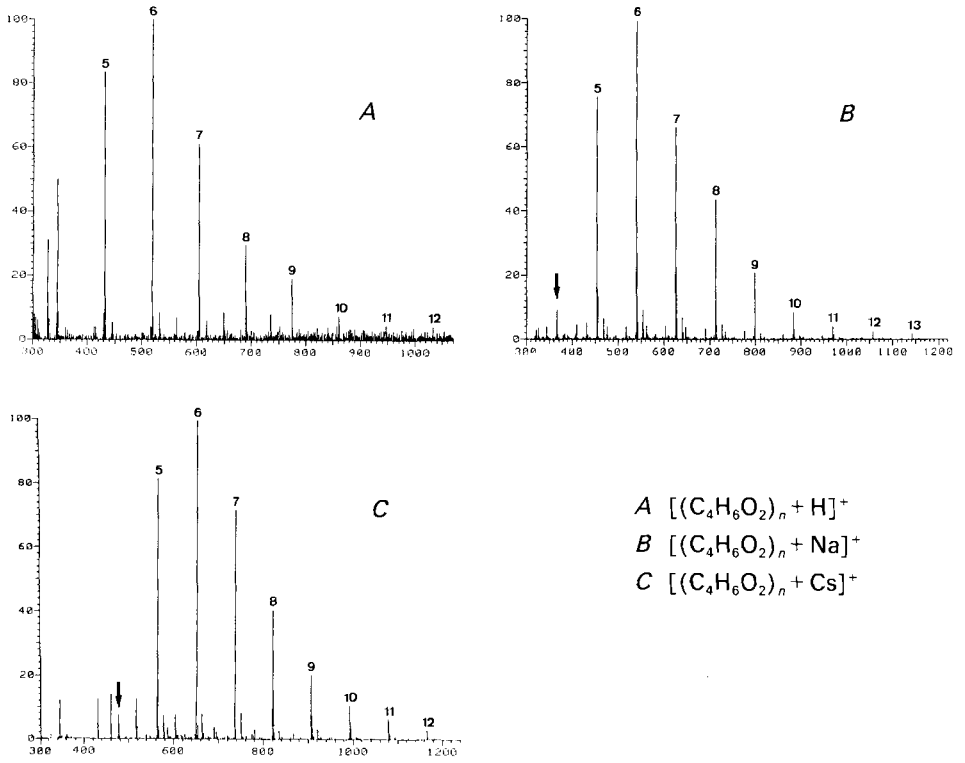


Fig. 2. *FAB-MS of* **1**, **2**, *and* **3**. Homogeneous MeOH/tetraethyleneglycol (TEG) solvent matrix, monoprotonated ions $M\mathrm{H}^+$ of the pentamer (mol.wt. 430), hexamer (516), and heptamer (602). The fragmentation pattern is interpreted for the pentamer **1**·H^+ in the *Exper. Part.*

by the octamer and that these two cyclic oligomers have identical R_f values. Indeed, the $M\mathrm{H}^+$ and the $M\mathrm{Na}^+$ FAB-MS of a freshly chromatographed, but not recrystallized sample of what appeared to be pure heptamer (one spot on TLC) showed that it contained *ca.* 40% of the octamer *(Fig. 5)*. The heptamer **3** crystallized from such mixtures and can be obtained in very pure form by multiple recrystallization, while we have so far not been able to isolate the pure octamer.

A lactonization experiment[8]) with the (*S*)-3-hydroxybutanoic acid [7] of 92% ee gave the enantiomers *ent*-**1**, *ent*-**2**, and *ent*-**3** in similar yields, while not a single pure product could be isolated from a cyclization experiment with the racemic hydroxy acid or with (*R*)-4,4,4-trifluoro-3-hydroxybutanoic acid [19]. Also, *Shanzer*'s method of using a tetra-

[8]) In an attempt to use a more readily available trichlorobenzoic acid (the 2,4,6-isomer must be prepared from trichlorobenzene [17]), we oxidized 2,3,6-trichlorobenzaldehyde (a development product of the *Hoechst AG*, D–Frankfurt-Hoechst) to the acid (83% by *Sargent*'s method [18]) and converted it to the acyl chloride. This gave 50% lower yields in the *Yamaguchi* procedure than the 2,4,6-isomer when applied to (*R*)-3-hydroxybutanoic acid.



	Relative Intensity [%] of Oligomers (n)[a]								
n	5	6	7	8	9	10	11	12	13
A	29.7	35.1	21.2	10.4	2.3	1.4	–	–	–
B	23.5	31.1	20.5	13.4	6.4	2.7	1.2	0.5	05
C	24.7	29.9	21.4	12.0	6.1	3.1	1.9	1.0	–

[a]) Distribution in % numeric values. The similarity of the numbers from the Na^+ and from the Cs^+ experiment shows that there is no specific complexation between any of the oligomers and one of these ions.

Fig. 3. *Cyclic-oligomer distribution of protonated and cationized molecular ions in the crude lactonization* (25°) *mixtures* by FAB-MS. *A)* MS in MeOH/TEG 1:1; *B)* MS in MeOH/TEG spiked with NaI (1:1); *C)* MS in MeOH/TEG spiked with CsI (1:1). Mass ranges covering molecular ions of oligomers from n = 1–12 are displayed. In each case, ion intensities are normalized to the most abundant oligomer, *i.e.* the hexamer (M = 516).

oxadistannacyclodecane [12] for macrocyclization was applied to *rac*-β-butyrolactone, to (*R*)-3-hydroxybutanoic acid and its methyl ester, and to PHB without success (no cyclic oligomers at all, by FAB-MS). Finally, we were not able to detect any oligomers in the reaction mixture obtained from (*R*)-3-hydroxybutanoic acid under *Mitsunobu*'s esterification/lactonization conditions [20].

We hoped to increase our chance of obtaining the macrotetrolide derived from hydroxybutanoic acid by systematically preparing the open-chain dimer **4** and tetramer **5** *(Scheme 2)* for directed cyclisations.

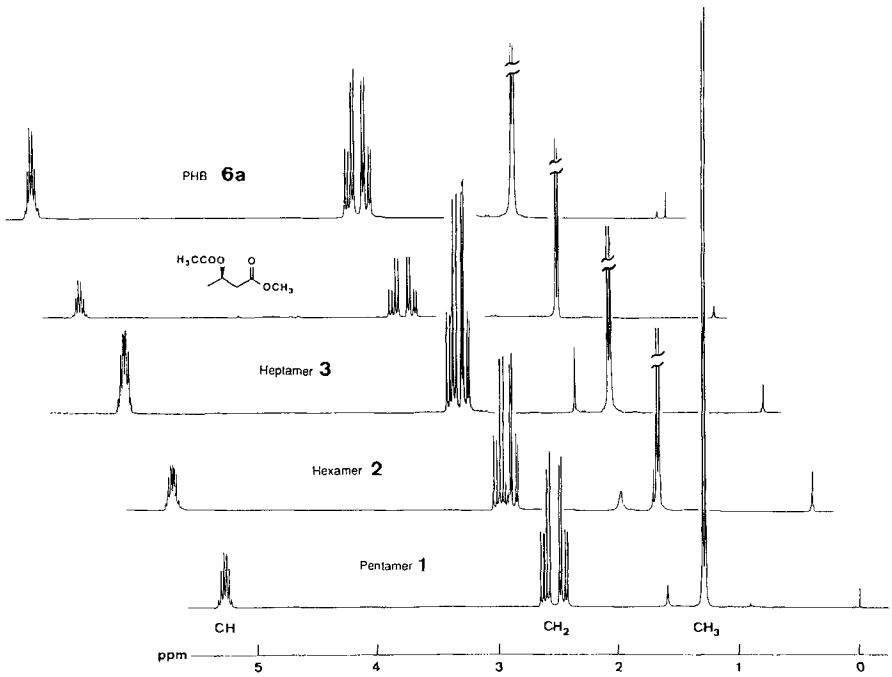


Fig. 4. *300-MHz ^{1}H-NMR Spectra* ($CDCl_3$) *of the cyclic oligomers* **1**, **2**, *and* **3**, *compared with the acetoxy methyl ester and the polymer PHB.* The spectra are recorded at room temperature; concentrations of *ca.* 10 mg/ml. In the spectrum of the acetoxy ester, the CH_3CO and CH_3O signals have been deleted. Obviously, there is a striking similarity, almost superimposability of these spectra. This indicates that the average conformations around the $CH_3CH(OR)CH_2COOR'$ moieties of these species are very similar (for a published ^{1}H-NMR spectrum of PHB, see [21b]).

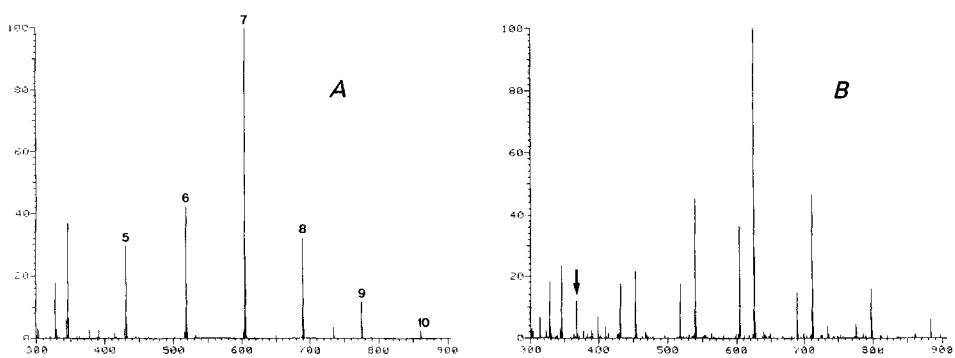


Fig. 5. *FAB-MS of a crude heptamer fraction from column chromatography. A)* In MeOH/TEG 1:1; *B)* in MeOH/TEG, spiked with NaI. Analysis of both spectra gives a content of *ca.* 11% of pentamer, 19% of hexamer, 44% of heptamer, 18% of octamer, 6% of nonamer, and 2% of decamer (average values from *A* and *B*).

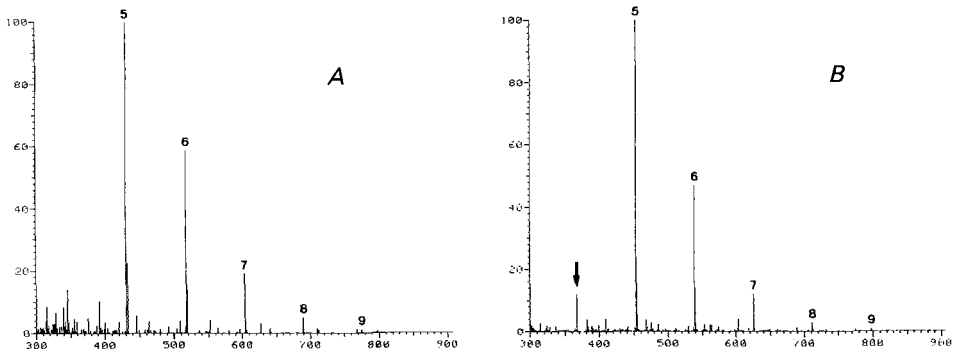
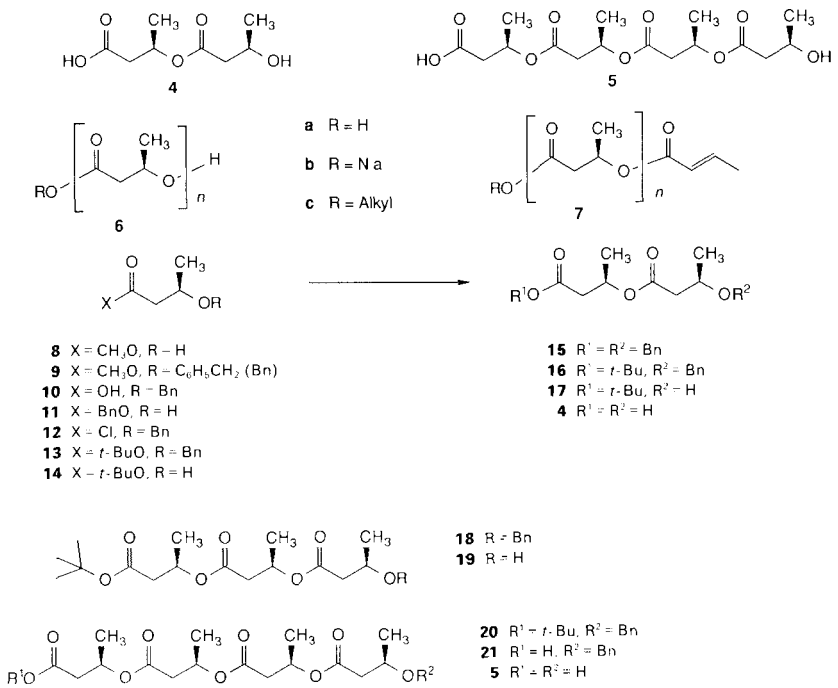


Fig. 6. *FAB-MS of the crude product mixture obtained from 3-hydroxybutanoic acid at 110°. A) MH^+; B) MNa^+* ions. The oligomer distribution is: pentamer 59%, hexamer 30%, heptamer 9%, octamer 1.5%, nonamer < 0.5% (average from MH^+ and MNa^+ spectrum).

Open-chain oligomers of (*R*)-3-hydroxybutanoic acid have been obtained from the biopolymer PHB by partial degradation/depolymerization or from monomeric derivatives by oligomerization. Thus, oligomers from the dimer to the icosamer have been identified by HPLC techniques [21a]. Depending upon the conditions of degradation, *i.e.* acidic hydrolysis or alcoholysis [22], alkaline saponification [23] or pyrolysis [24], the acidic end group of the oligomer may be a free acid (**6a** or **7a**), an ester (**6c** or **7c**), or a Na salt (**6b** or **7b**), and the other end of the oligomer may carry a free OH-group or a

Scheme 2



crotyl-ester group. Our attempts to identify oligomeric esters during depolymerization of PHB with $Ti(OR)_4$ in alcohol [14a] [25] [26] were unsuccessful. Also, depolymerization with pig-liver esterase in aqueous alcohols and in non-aqueous solvents did not take place. Oligomer formation has been observed when monomeric hydroxybutanoic esters are stored for longer periods of time, especially in the presence of traces of acid or base [7]. Also, higher-molecular-weight esters are formed [27] from the β-lactone [14c] [28] of 3-hydroxybutanoic acid. There is an interest in oligomers of the type **6b**, because it is known that sodium (*R*)-3-hydroxybutanoate can replace glucose as brain nutrient [29], and it is hoped that a salt of an oligomer would still do so, while having a higher, more favorable hydroxy acid/sodium ratio.

The intermediates of our preparation of the dimer **4** and tetramer **5** – in a peptide-synthesis fashion with repeating identical reaction steps – are the compounds **8**–**21**. The methyl ester **8** of 3-hydroxybutanoic acid [14a] was benzylated with *O*-benzyl trichloroacetimidate [30] (→**9**), saponified to the acid **10**, and coupled with benzyl 3-hydroxybutanoate **11** by simple TsOH-catalyzed esterification to give the dibenzylated dimer **15**. This was deprotected by Pd/C-catalyzed hydrogenation to yield the desired hydroxy acid **4** (*ca.* 30% overall yield from **8**). For the synthesis of the tetramer **5**, the (benzyloxy)acyl chloride **12** (from **10** + $SOCl_2$) was the most important reagent. It could not be purified by distillation without decomposition, so that we employed crude **12** in all subsequent experiments, such as the reaction with *tert*-butyl (*R*)-3-hydroxybutanoate (**14**). The *tert*-butyl-ester group served as protection of the acid end in the tetramer to be prepared: By a series of three debenzylation/acylation sequences with H_2/Pd and the acyl chloride **12** (**13**→**14**→**16**, **16**→**17**→**18**, and **18**→**19**→**20**), the tetramer with a benzyl protection on the alcohol end was obtained (total yield **13**→**20**: *ca.* 30%). Deprotection first of the acid group with CF_3COOH (→**21**), and then of the OH group (H_2/Pd) gave the desired tetramer **5**. The intermediates in these reaction sequences were purified by chromatography and characterized by ^{1}H-NMR, IR, and mass spectroscopy.

When subjected to *Yamaguchi*'s macrolactonization conditions, the dimer **4** gave crotonic acid (21%) and the cyclic oligomers **1** (6%, pentamer) and **2** (5%, hexamer) as the only identified products[9]). Likewise, we did not detect the cyclic tetramer (**B** in *Fig. 1*) when subjecting the open-chain tetramer to the lactonization conditions: the reaction was followed by TLC, the starting material did not seem to disappear, the spots of the cyclic oligomers **1**–**3** were not seen.

C) Discussion of the Results – Open Questions. – The rather sharp distribution of cyclic oligomers with 20-, 24-, and 28-membered rings formed from 3-hydroxybutanoic acid under macrolactonization conditions, the missing 16-membered-ring tetramer, the close resemblance of the NMR spectra of the monomer, of the oligomers, and of the polymer, and the non-selective complexation of Na^+ and Cs^+ ions by these macrocycles in the gas phase raise more questions than they answer.

The preference for certain ring sizes is reminiscent of the modifications of elemental sulfur: only within certain temperature ranges do cyclic forms prevail. Also, there is a so-called ceiling temperature in polymerizations above which no more polymer is formed; ring/chain equilibria may be established, or ring formation may be kinetically

[9]) No cyclic oligomers were detected upon treatment of the dimer **4** under *Mitsunobu*'s macrolactonization conditions, which might have produced a cyclic tetramer from two (*R*)- and two (*S*)-3-hydroxybutanoic acids.

controlled [31] [32]. So far, we have carried out only one macrolactonization experiment with 3-hydroxybutanoic acid at higher temperature: 110° instead of the usual room temperature[7]). As can be seen from *Fig. 6*, there is a shift of the cyclic-oligomer distribution towards the lower-molecular-weight species, the pentamer predominates. In principle, this result may be caused by kinetic or by thermodynamic control. The observation that the dimeric hydroxy acid **4** gave some cyclic pentamer suggests that ester bonds may not only be made but also be cleaved under the reaction conditions.

Measurements of the temperature-dependant NMR spectra of the oligomers **1–3**, X-ray crystal structure analyses, molecular modelling studies, and cyclizations under a variety of conditions are being undertaken in our laboratories, in order to answer the open questions posed by our observation.

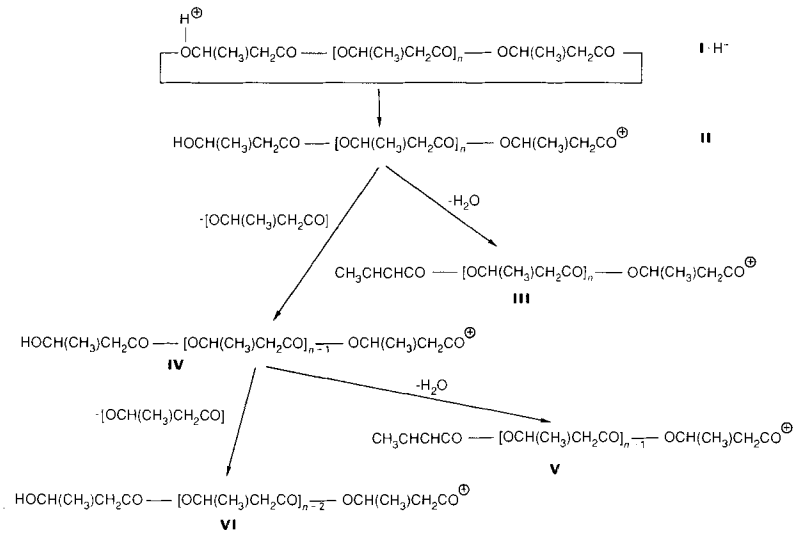
Experimental Part

General. All solvents for reactions were of *purissimum* quality. Unless otherwise stated, org. extracts were dried with $MgSO_4$ and evaporated by using a rotary evaporator. TLC: *Merck-DC-F$_{254}$* plates; detection by UV light, I_2, or spraying with a soln. of 25 g of phosphormolybdic acid, 10 g of $Ce(SO_4)_2 \cdot 4\,H_2O$, 60 ml of conc. H_2SO_4, and 940 ml of H_2O. Flash chromatography (FC): *Merck* silica gel (mesh size 0.040–0.063). Specific rotations: *Perkin-Elmer-241* polarimeter; $CHCl_3$ solns. at 25°; *c* in g/100 ml. M.p.: *Büchi/Tottoli* melting point apparatus; uncorrected. IR spectra: *Perkin-Elmer-297* spectrometer; KBr discs or $CHCl_3$ soln. ^{1}H- and ^{13}C-NMR spectra: *Varian EM-390* (90 MHz) or *Bruker-WM-300* (300 MHz) instrument; TMS as internal standard, $CDCl_3$ solns. Molecular-weight determinations were carried out using the apparatus described in [33]: MS: 70 eV; *Hitachi-Perkin-Elmer-RMV-6M* instrument; *m/z* values with relative intensities (%) in parentheses. FAB-MS: modified *Finnigan-AMD/MAT-312* spectrometer equipped with a laminated, extended magnet and analyzer (*AMD Intectra*, Beckeln, FRG): spectra were obtained with a combined EI/FD/FAB ion source, employing a self-constructed, adjustable direct insertion probe with *ca.* 2-mm^2 stainless-steel, thermostated target tip; a Cs thermoionic primary particle source according to the design by *Alberth et al.* [34] was used at a perpendicular ion-source port, with an incident angle of *ca.* 60°; other instrumental conditions were as previously described [35]; acquisition of spectra was obtained by the *Finnigan*-SS-200 data system at a resolution of 2000 (10%) and a scan rate of 16 sec decade^{-1}; *ca.* 10 single scans were averaged using a spectra averaging routine by the *SSX* software as previously described [36]; FAB cluster ion spectra of a soln. of CsI in glycerol were used for mass calibration up to *m/z* 4000 [36]; sample solns. were constituted from solns. of oligomers of *ca.* 2–5 μg/μl in MeOH, and 1-μl aliquots were admixed carefully to 1 μl of tetraethylene glycol on the FAB target, to yield a completely homogeneous matrix; alkali-cationization experiments were carried out by spiking *ca.* 0.3 μg/μl of NaI or CsI to the TEG matrix. Linked-scan metastable experiments: B/E linked scan daughter ion spectra were obtained from the same solns., and FAB conditions described above, using the *BMS* metascan unit of the *MAT-312* instrument; spectra were obtained by manual mass calibration, and recorded at a scan rate of 8 mu/sec with a UV oscillographic recorder; from these data the stepwise fragmentation pathway depicted in *Scheme 3* is clearly derived.

Pentamer **1**, *Hexamer* **2**, *and Heptamer* **3**. To a stirred soln. of (*R*)-3-hydroxybutanoic acid (0.5 g, 4.8 mmol) in 10 mmol THF at 0° was slowly added Et_3N (0.87 ml, 6.2 mmol) and 2,4,6-trichlorobenzoyl chloride (0.67 ml, 4.8 mmol). After 30 min, the ice-bath was removed and the temp. allowed to rise within 1.5 h from 0° to r.t. The suspension was filtered and the filtrate diluted with 10 ml toluene. This soln. was added over 4 h (with a motor-driven syringe) under Ar to a stirred soln. of 4-(*N,N*-dimethylamino)pyridine in toluene (200 ml). Stirring was continued for a further 30 min. The mixture was diluted with 100 ml of Et_2O and washed with 100-ml portions of 1N HCl, sat. $NaHCO_3$ soln., and sat. NaCl soln. Workup gave 359 mg of a half crystalline, half oily crude product. FC (Et_2O/petroleum ether 7:3, 110 g, of silica gel) gave fractions of **1** (R_f 0.29), **2** (R_f 0.22), and **3** (R_f 0.17). Three recrystallisations of each product ($CHCl_3$/hexane) gave pure, sharply melting products **1**, **2** and **3**.

(4R,8R,12R,16R,20R)-4,8,12,16,20-Pentamethyl-1,5,9,13,17-pentaoxacycloicosane-2,6,10,14,18-pentone (**1**): 62 mg (15%). M.p. 102.5–103.5°. $[\alpha]_D^{r.t.} = +6.7°$ (*c* = 1.03). IR (KBr): 2995*m*, 2960*w*, 1740*s*, 1450*w*, 1430*w*, 1380*m*, 1305*s*, 1260*s*, 1205*s*, 1185*s*, 1140*m*, 1105*s*. ^{1}H-NMR (300 MHz): 5.22–5.33 (*m*, 5 CH_3CHO); 2.60 (*dd*, *J* = 7.8, 15.2, 5 H, 5 CH_2); 2.45 (*dd*, *J* = 5.6, 15.2, 5 H, 5 CH_2); 1.31 (*d*, *J* = 6, 5 CH_3). FAB-MS: 431 (100, $[M + 1]^+$), 345 (6, $[M - 85]^+$), 327 (2), 259 (8), 241 (8), 195 (61, matrix ion), 173 (11), 155 (52). Anal. calc. for $C_{20}H_{30}O_{10}$: C 55.81, H 7.02; found: C 55.45, H 6.67. Mol.wt. (osmom.): 410.8.

Scheme 3[a])



[a]) Protonation of the oligomer **I**, cleavage of an ester bond to the open-chain acylium ion **II** and loss of H_2O leads to **III**. Alternatively, loss of an $[OCH(CH_3)CH_2CO]$ moiety leads to **IV**, which subsequently can undergo analogous fragmentations (→**V**, **VI**) *etc.* (see also *Fig. 2*, pentamer).

(4R,8R,12R,16R,20R,24R)-4,8,12,16,20,24-Hexamethyl-1,5,9,13,17,21-hexaoxacyclotetracosane-2,6,10,14,18,22-hexone (**2**): 68 mg (16%). M.p. 112.0–113.5°. $[\alpha]_D^{r.t.} = +7.3°$ ($c = 1.09$). IR (KBr): 2995*m*, 2940*w*, 1725*s*, 1460*w*, 1400*m*, 1370*m*, 1305*s*, 1203*s*, 1195*s*, 1140*m*, 1110*m*, 1055*m*, 975*m*. ^{1}H-NMR (300 MHz): 5.25–5.36 (*m*, 6 CH_3CHO); 2.60 (*dd*, $J = 8.5$, 15.8, 6 H, 6 CH_2); 2.45 (*dd*, $J = 4.8$, 15.8, 6 H, 6 CH_2); 1.27 (*d*, $J = 6$, 6 CH_3). FAB-MS: 517 (100, $[M + 1]^+$), 431 (6), 345 (13), 327 (6), 259 (15), 241 (24). Anal. calc. for $C_{24}H_{36}O_{12}$: C 55.81, H 7.02; found: C 55.47, H 7.31. Mol.wt. (osmom.): 501.9.

(4R,8R,12R,16R,20R,24R,28R)-4,8,12,16,20,24,28-Heptamethyl-1,5,9,13,17,21,25-heptaoxacyclooctacosane-2,6,10,14,18,22,26-heptone (**3**): 79 mg (19%). M.p. 118.0–119.2°. $[\alpha]_D^{r.t.} = +1.2°$ ($c = 0.93$). IR (KBr): 2995*m*, 2970*w*, 1745*vs*, 1455*w*, 1390*m*, 1305*s*, 1270*m*, 1190*s*, 1135*m*, 1100*m*, 1060*s*, 980*m*. ^{1}H-NMR (300 MHz): 5.23–5.34 (*m*, 7 CH_3CHO); 2.60 (*dd*, $J = 8.5$, 15.8, 7 H, 7 CH_2); 2.49 (*dd*, $J = 5.0$, 15.8, 7 H, 7 CH_2); 1.27 (*d*, $J = 6.3$, 7 CH_3). FAB-MS: 603 (100, $[M + 1]^+$), 517 (2), 431 (5), 345 (10), 327 (7), 259 (10), 241 (20). Anal. calc. for $C_{28}H_{42}O_{14}$: C 55.81, H 7.02; found: C 55.64, H 7.05. Mol.wt. (osmom.): 654.3.

The above described procedure with (*S*)-3-hydroxybutanoic acid (0.5 g, 4.8 mmol) gave, after FC (Et_2O/petroleumether 7:3), *ent*-**1**, *ent*-**2**, and *ent*-**3**.

ent-**1**: 54 mg (13%). M.p. 109–110°. $[\alpha]_D^{r.t.} = -3.7°$ ($c = 0.9$). IR ($CHCl_3$): 2990*m*, 1740*vs*, 1380*m*, 1305*m*, 1190*s*, 1055*m*. ^{1}H-NMR (90 MHz): 5.02–5.38 (*m*, 5 CH_3CHO); 2.35–2.60 (*m*, 5 CH_2); 1.27 (*d*, $J = 6$, 5 CH_3). MS: 431 (0.2, $[M + 1]^+$), 345 (3), 327 (0.5), 259 (6), 241 (7), 173 (17), 155 (100). Mol.wt. (osmom.): 451.5.

ent-**2**: 54 mg (13%). M.p. 109–110°. $[\alpha]_D^{r.t.} = -8.8°$ ($c = 0.85$). IR ($CHCl_3$): 2991*m*, 1740*vs*, 1381*m*, 1305*m*, 1190*m*, 1058*m*. ^{1}H-NMR (90 MHz): 5.05–5.45 (*m*, 6 CH_3CHO); 2.38–2.60 (*m*, 6 CH_2); 1.25 (*d*, $J = 7$, 6 CH_3). MS: 517 (0.8, $[M + 1]^+$), 516 (2, M^+), 431 (6), 259 (29), 241 (3). Mol.wt. (osmom.): 534.3.

ent-**3**: 27 mg (7%). M.p. 110–111°. $[\alpha]_D^{r.t.} = -1.1°$ ($c = 0.36$). IR ($CHCl_3$): 2990*m*, 1740*s*, 1380*m*, 1305*m*, 1190*s*, 1055*m*. ^{1}H-NMR (90 MHz): 5.02–5.35 (*m*, 7 CH_3CHO); 2.38–2.60 (*m*, 7 CH_2); 1.25 (*d*, $J = 6$, 7 CH_3). MS: 603 (0.3, $[M + 1]^+$), 602 (0.6, M^+), 517 (2), 431 (4), 345 (11), 327 (9), 259 (3), 241 (4). Mol.wt. (osmom.): 601.0.

Methyl (R)-3-(Benzyloxy)butanoate (**9**). To a soln. of *methyl (R)-3-hydroxybutanoate* (**8**; 4.7 g, 40 mmol) and *O*-benzyltrichloroacetimidate (11.1 g, 44 mmol) in cyclohexane/CH_2Cl_2 was added trifluormethansulfonic acid (2 ml) with stirring overnight at r.t. The resulting dark orange suspension was washed with a sat. $NaHCO_3$ soln., dried, diluted with 200 ml of hexane, and evaporated. The white precipitate was filtered off and the filtrate evaporated again. Bulb-to-bulb distillation of the yellow oil gave 7.2 g (86%) of **9** as a slightly yellow viscous liquid. B.p. 175°/0.21 Torr. IR (film): 3030*w*, 2975*w*, 1740*s*, 1450*m*. ^{1}H-NMR (90 MHz): 7.32 (*s*, 5 arom. H); 4.56 (*s*,

$PhCH_2O$); 3.89–4.18 (*m*, H–C(3)); 3.65 (*s*, CH_3O); 2.25–2.85 (*m*, 2 H–C(2)); 1.28 (*d*, $J = 6$, CH_3). MS: 208 (1.3, M^+), 107 (48), 102 (32), 91 (100), 87 (26), 79 (13), 65 (12), 43 (18).

(R)-3-(Benzyloxy)butanoic Acid (**10**). To **9** (2.08 g, 10 mmol) at 0° was added 1N aq. KOH (12 ml, 12 mmol). After 1 h, the ice-bath was removed and the mixture stirred for further 2 h at r.t. The soln. was extracted with 20 ml of Et_2O, the aq. phase was brought to pH 2 with 1N HCl and extracted with 3 × 30 ml portions of Et_2O. Evaporation of the solvent gave 1.8 g (93%) of **10**. ^{1}H-NMR (90 MHz): 11.1 (*s*, COOH); 7.29 (*s*, 5 arom. H); 4.56 (*s*, $PhCH_2O$); 3.78–4.21 (*m*, H–C(3)); 2.31–2.87 (*m*, 2 H–C(2)); 1.30 (*d*, $J = 6$, CH_3).

Benzyl (R)-3-Hydroxybutanoate (**11**). A soln. of (*R*)-3-hydroxybutanoic acid (2.6 g, 25 mmol), benzyl alcohol (27 g, 250 mmol), benzene (125 ml), and a cat. amount of TsOH was heated under reflux overnight using a H_2O trap. The solvent was removed and the residue diluted with 20 ml of Et_2O, washed with a 5% $NaHCO_3$ soln., dried, and evaporated. FC (Et_2O/hexane 3:7) gave 3.77 g (71%) of **11**. IR (film): 3600–3200*m* (br.), 3060*w*, 2980*m*, 1725*s*, 1450*m*. ^{1}H-NMR (90 MHz): 7.30 (*s*, 5 arom. H); 5.10 (*s*, $PhCH_2O$); 4.0–4.32 (*m*, H–C(3)); 3.20 (*s*, OH); 2.46 (*d*, $J = 6$, 2 H–C(2)); 1.08–1.32 (*d*, $J = 6$, CH_3). MS: 194 (5, M^+), 108 (39), 107 (60), 105 (18), 92 (17), 91 (100), 79 (18), 45 (17).

(3R)-3-{[(3'R)-3'-(Benzyloxy)butanoyl]oxy}butanoate (**15**). Similar to the synthesis of **11**, **10** (1.28 g, 6.6 mmol), **11** (1.28 g, 6.6 mmol), benzene (15 ml), and a cat. amount of TsOH gave 2.3 g (94%) of crude product. FC (Et_2O/hexane 2:8) gave 1.0 g (41%) of **15**. IR (film): 3060*w*, 2980*w*, 1735*s*, 1495*w*, 1451*m*, 1375*m*, 1180*s*. ^{1}H-NMR (90 MHz): 7.35 (*s*, 10 arom H); 5.18–5.50 (*m*, H–C(3)); 5.09 (*s*, $PhCH_2OOC$); 4.50 (*s*, $PhCH_2O$); 3.72–4.12 (*m*, H–C(3')); 2.12–2.80 (*m*, 2 H–C(2), 2 H–C(2')); 1.05–1.40 (*m*, 2 CH_3).

(3R)-3-{[(3'R)-3'-Hydroxybutanoyl]oxy}butanoic Acid (**4**). A suspension of **15** (2 g, 5.3 mmol) and 600 mg of Pd/C (10%) in 100 ml of abs. EtOH was stirred under H_2 (balloon) for 6 h. Filtration through *Celite*, evaporation, and bulb-to-bulb distillation gave 850 mg (84%) of **4** as a slightly yellow liquid. B.p. 160°/0.2 Torr. $[\alpha]_D^{r.t.} = -33.7°$ ($c = 1.19$). IR (film): 3650–2500*m* (br.), 2980*w*, 1725*vs*, 1380*w*. ^{1}H-NMR (90 MHz): 6.74–7.45 (br. *s*, OH, COOH); 5.10–5.55 (*m*, H–C(3)); 3.95–4.40 (*m*, H–C(3')); 2.25–2.70 (*m*, 2 H–C(2), 2 H–C(2')); 1.05–1.40 (*m*, 2 CH_3). MS: 115 (29), 105 (13), 87 (48), 86 (33), 69 (61), 45 (58), 43 (100), 41 (46).

(R)-3-(Benzyloxy)butanoyl Chloride (**12**). A soln. of **10** (1.0 g, 5.1 mmol) and $SOCl_2$ (0.56 ml, 7.7 mmol) was stirred overnight. The excess $SOCl_2$ was removed under high vacuum: 1.0 g (92%) of crude **12**. ^{1}H-NMR (90 MHz): 7.35 (*s*, arom. H); 4.58 (*s*, $PhCH_2O$); 3.89–4.30 (*m*, H–C(3)); 2.80–3.40 (*m*, 2 H–C(2)); 1.20–1.40 (*d*, $J = 6$, CH_3).

tert-*Butyl (R)-3-(Benzyloxy)butanoate* (**13**). To a soln. of *t*-BuOH (6.6 ml, 32.3 mmol) and Et_3N (3.0 ml, 21.2 mmol) at 0° was added a soln. of crude **12** (3.0 g, 14.1 mmol) in 6 ml of $CHCl_3$. The mixture was refluxed for 3 h, poured onto ice, and diluted with 5 ml of Et_2O. The org. phase was separated, washed (sat. NaCl soln.), dried, and evaporated. The crude product (2.4 g, 68%) was purified by FC (Et_2O/hexane 1:9) giving 1.8 g (51%) of **13**. IR (film): 2975*s*, 1725*s*, 1450*m*, 1365*m*. ^{1}H-NMR (90 MHz): 7.28 (*s*, 5 arom. H); 4.51 (*s*, $PhCH_2O$); 3.80–4.18 (*m*, H–C(3)); 2.15–2.78 (*m*, 2 H–C(2)); 1.47 (*s*, *t*-Bu); 1.28 (*d*, $J = 6$, CH_3). MS: 193 (16, $[M - 57]^+$), 107 (95), 91 (100), 79 (15), 57 (34), 41 (16). Anal. calc. for $C_{15}H_{22}O_3$: C 71.93, H 8.86; found: C 71.80, H 8.93.

Debenzylation/Acylation Sequence to Prepare **20** *Starting with* **13**. *General Procedure 1 for Debenzylation.* A 0.09M soln. of the benzylated compound **13**, **16**, **18**, or **21** in abs. EtOH was stirred, with 20% Pd/C (10%) under H_2. The reaction was followed by TLC. After completion of the reaction (4–6 h), the suspension was filtered *(Celite)* and the product **14**, **17**, **19**, or **5**, resp., purified by bulb-to-bulb distillation.

tert-*Butyl (R)-3-Hydroxybutanoate* (**14**). *General Procedure 1* with **13** (4.5 g, 18 mmol) gave 2.6 g (93%) of **14**. B.p. 84°/0.1 Torr. IR (film): 3650*m* (br.), 2980*s*, 1725*s*, 1450*w*. ^{1}H-NMR (90 MHz): 3.90–4.34 (*m*, H–C(3)); 3.0–3.45 (br. *s*, OH); 2.22–2.52 (*m*, 2 H–C(2)); 1.45 (*s*, *t*-Bu); 1.20 (*d*, $J = 6$, CH_3). MS: 145 (2, $[M - 15]^+$), 87 (20), 59 (37), 57 (100), 43 (30).

tert-*Butyl (3R)-3-{[(3'R)-3'-Hydroxybutanoyl]oxy}butanoate* (**17**). *General Procedure 1* with **16** (2.5 g, 7.4 mmol) gave 1.8 g (98%) of **17**. B.p. 98°/0.16 Torr. IR (film): 3650–3100*m* (br.), 2980*s*, 1730*s*, 1450*w*. ^{1}H-NMR (90 MHz): 5.18–5.68 (*m*, H–C(3)); 4.0–4.40 (*m*, H–C(3')); 3.10–3.40 (br. *s*, OH); 2.30–2.69 (*m*, 2 H–C(2), 2 H–C(2')); 1.48 (*s*, *t*-Bu); 1.13–1.40 (*m*, 2 CH_3). MS: 191 (2, $[M - 55]^+$), 105 (23), 87 (60), 69 (58), 57 (100), 43 (30).

tert-*Butyl (3R)-3-{[(3'R)-3'-{[(3''R)-3''-Hydroxybutanoyl]oxy}butanoyl]oxy}butanoate* (**19**). *General Procedure 1* with **18** (2.1 g, 5 mmol) gave 1.67 g (quant.) of **19**. B.p. 120°/0.14 Torr. IR (film): 3650–3200*m* (br.), 2980*m*, 1740*vs*, 1455*w*. ^{1}H-NMR (90 MHz): 5.10–5.50 (*m*, H–C(3), H–C(3')); 4.02–4.38 (*m*, H–C(3'')); 2.31–2.68 (*m*, 2 H–C(2), 2 H–C(2'), 2 H–C(2''), OH); 1.43 (*s*, *t*-Bu); 1.04–1.38 (*m*, 3 CH_3). MS: 277 (1, $[M - 55]^+$), 191 (12), 173 (20), 155 (26), 105 (25), 87 (54), 69 (100), 57 (66), 43 (30).

(3R)-3-{[(3'R)-3'-{[(3''R)-3''-{[(3'''R)-3'''-Hydroxybutanoyl]oxy}butanoyl]oxy}butanoyl]oxy}butanoic Acid (**5**). *General Procedure 1* with **21** (650 mg, 1.4 mmol) gave 640 mg of crude **5**. $[\alpha]_D^{r.t.} = -14.1°$ ($c = 1.18$).

^{1}H-NMR (90 MHz): 6.31–6.53 (*m*, OH, COOH); 5.05–5.55 (*m*, H–C(3), H–C(3'), H–C(3")); 4.05–4.41 (*m*, H–C(3"')); 2.32–2.85 (*m*, 2 H–C(2), 2 H–C(2'), 2 H–C(2"), 2 H–C(2"')); 1.14–1.56 (*m*, 4 CH_3).

General Procedure 2 for Acylation. To the soln. of the hydroxy *tert*-butyl ester **14**, **17**, or **19** and an equimolar amount of Et_3N at 0°, the soln. of 1 equiv. of **12** in $CHCl_3$ was slowly added. The mixture was then heated under reflux for 3 h, and poured onto ice, the org. phase diluted with Et_2O, washed with sat. NaCl soln., dried, and evaporated. The product **16**, **18**, or **20** was purified by FC.

tert-*Butyl 3-{[(3'R)-3'-(Benzyloxy)butanoyl]oxy}butanoate* (**16**). *General Procedure 2* with **14** (1.8 g, 11.2 mmol) and **12** (2.4 g, 11.2 mmol) gave, after FC (Et_2O/hexane 2:8), 2.61 g (71%) of **16**. IR (film): 2980*m*, 1732*vs*, 1450*w*. ^{1}H-NMR (90 MHz): 7.3 (*s*, 5 arom. H); 5.05–5.45 (*m*, H–C(3)); 4.50 (*s*, PhCH_2O); 3.81–4.20 (*m*, H–C(3')); 2.18–2.80 (*m*, 2 H–C(2), 2 H–C(2')); 1.40 (*s*, *t*-Bu); 1.15–1.35 (*m*, 2 CH_3). MS: 279 (7, $[M-57]^+$), 174 (20), 107 (50), 91 (100), 87 (28), 69 (40), 57 (40), 41 (38).

tert-*Butyl (3R)-3-{[(3'R)-3'-{[(3"R)-3"-(Benzyloxy)butanoyl]oxy}butanoyl]oxy}butanoate* (**18**). *General Procedure 2* with **17** (1.81 g, 7.4 mmol) and **12** (1.57 g, 7.4 mmol) gave, after FC (Et_2O/hexane 1:4), 2.26 g (72%) of **18**. IR (film): 2980*m*, 1735*vs*, 1450*w*. ^{1}H-NMR (90 MHz): 7.30 (*s*, 5 arom. H); 5.0–5.40 (*m*, H–C(3), H–C(3')); 4.51 (*s*, PhCH_2O); 3.80–4.21 (*m*, H–C(3")); 2.20–2.85 (*m*, 2 H–C(2), 2 H–C(2'), 2 H–C(2")); 1.45 (*s*, *t*-Bu); 1.10–1.39 (*m*, 3 CH_3).

tert-*Butyl (3R)-3-{[(3'R)-3'-{[(3"R)-3"-{[(3"'R)-3"'-(Benzyloxy)butanoyl]oxy}butanoyl]oxy}butanoyl]oxy}butanoate* (**20**). *General Procedure 2* with **19** (1 g, 3 mmol) and **12** (0.63 g, 3 mmol) gave, after FC (Et_2O/hexane 2:3), 1.04 g (68%) of **20**. IR (film): 2980*m*, 1735*vs*, 1450*w*. ^{1}H-NMR (90 MHz): 7.3 (*s*, 5 arom. H); 5.02–5.50 (*m*, H–C(3), H–C(3'), H–C(3")); 4.55 (*s*, PhCH_2O); 3.82–4.20 (*m*, H–C(3"')); 2.20–2.88 (*m*, 2 H–C(2), 2 H–C(2'), 2 H–C(2"), 2 H–C(2"')); 1.41 (*s*, *t*-Bu); 1.3 (*d*, $J = 7$, 4 CH_3).

(3R)-3-{[(3'R)-3'-{[(3"R)-3"-{[(3"'R)-3"'-(Benzyloxy)butanoyl]oxy}butanoyl]oxy}butanoyl]oxy}butanoic Acid (**21**). To a soln. of **20** (950 mg, 1.9 mmol) in 8 ml of CH_2Cl_2 was added a catalytic amount of CF_3COOH and stirred 72 h at r.t. The solvent was evaporated, and FC (Et_2O/hexane 7:3) gave 670 mg (80%) of **21**. ^{1}H-NMR (90 MHz): 9.30–9.85 (br. *s*, COOH); 7.28 (*s*, 5 arom. H); 5.0–5.50 (*m*, H–C(3), H–C(3'), H–C(3")); 4.50 (*s*, PhCH_2O); 3.78–4.20 (*m*, H–C(3"')); 2.21–2.86 (*m*, 2 H–C(2), 2 H–C(2'), 2 H–C(2"), 2 H–C(2"')); 1.10–1.54 (*m*, 4 CH_3).

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