104. Synthesis of Linear Oligomers of (*R*)-3-Hydroxybutyrate and Solid-State Structural Investigations by Electron Microscopy and X-Ray Scattering

by **Dieter Seebach***, **H. Michael Bürger**[1]), **Hans-Martin Müller**[1]), **Urs D. Lengweiler**[1]), and **Albert K. Beck**

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

and **Kathy E. Sykes**, **Phil A. Barker**, and **Peter J. Barham***

H. H. Wills Physics Laboratory, University of Bristol, Tyndall Avenue, GB-Bristol BS8 1TL

Dedicated to Prof. Dr. *H. Ringsdorf* on the occasion of his 65th birthday

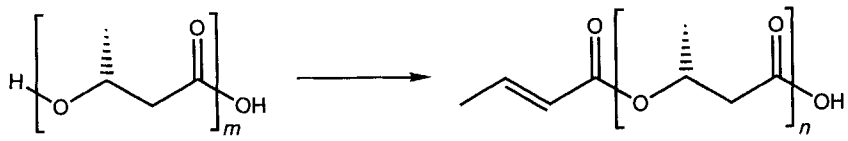
(9.II.94)

Repetitive treatment of the biopolymer P(3-HB) (molecular weight $> 10^5$ Dalton, *storage* or *s*-P(3-HB)), with lithium hexamethyl disilazanid (LHMDS) at $-70°$ in THF leads to a mixture of oligomers with increasingly sharp distribution around a 15-, 30-, and 45mer. Discrete fragments are also isolated when P(3-HB) is heated under reflux (89°) in neat Et_3N. Linear oligo(3-HB) derivatives (**3**–**7**) containing up to 96 3-HB units are synthesized using an exponential segment-coupling strategy. These oligomers are used to calibrate size-exclusion chromatography columns for the analysis of oligo(3-HB) samples from the different sources. The linear oligo-(3-HB) derivatives also served as a model with respect to the physical properties of high molecular weight P(3-HB) and were investigated as such by transmission electron microscopy (TEM) and by small- and wide-angle X-ray scattering (SAXS and WAXS). The thicknesses of the lamellar crystallites (long periods) formed by the 8mer, 16mer, and 32mer, are *ca.* 26, 52, and 53 Å, respectively, indicating that the 32mer molecules are folded once, very tightly, into a 'hair-pin'-type conformation. High-molecular-weight P(3-HB), which was crystallized in a similar way, also has a lamellar crystallite thickness of *ca.* 50–65 Å. Thus, the treatment of P(3-HB) with LHMDS at low temperature causes etching of the amorphous regions, an effect well known in polymer science for studying the regularity of chain folding. The *ca.* 50-Å packing within the tight folds of P(3-HB) is discussed in view of its possible function in ion transport through cell membranes.

Polyhydroxybutyrate (P(3-HB)) is the prototype of a class of biopolymers [1] [2] called polyhydroxyalkanoates (PHA; for review articles, see [3–5]). They are used by microorganisms as a means to store energy and reductase equivalents and as a carbon source (high-molecular-weight *s*-P(3-HB), $> 10^5$ Dalton). A copolymer of P(3-HB) and P(3-HV) (V = valerate) is produced by fermentation and sold as a biodegradable and biocompatible plastic material under the trade name *BIOPOL* [6]. Low-molecular-weight P(3-HB) (100–200 units) was found in the membranes of prokaryotic and eukaryotic cells. It forms *complexes*, for instance, with calcium polyphosphate and albumin (*c*-P(3-HB)) [7]. The structure and function of *c*-P(3-HB) are greatly in debate, concerning suggestions including stabilizing proteins to a Ca^{2+} polyphosphate (PPi), and acting as a DNA channel through lipid membranes [7]. We have embarked on an investigation to obtain information on the structure and properties of oligo(3-HB) derivatives [3].

[1]) Part of the Ph. D. Theses of *H. M. B.* (ETH-Zürich, Dissertation No. 10436, 1993), *H.-M. M.* (ETH-Zürich, Dissertation No. 9685, 1992), and of the projected dissertation of *U. D. L.*

1. Introduction – A Puzzling Observation. – In a previous paper from the ETH laboratory [8], it has been shown that treatment of poly[(*R*)-3-hydroxybutanoate] (**1**; P(3-HB)), with lithium amide bases in THF at dry-ice temperature leads, after aqueous workup, to partial but not total degradation, with formation of oligomers **2** bearing crotyl groups at the hydroxy terminus[2]). The molecular weights of these oligomers were established by NMR-spectroscopic end-group determination (methyl esters), by osmometric measurements in $CHCl_3$, by gel-permeation chromatography (GPC, polystyrene standard), and by plasma-desorption mass spectroscopy. These methods gave somewhat conflicting results, with the size-exclusion chromatography giving particularly high values of molecular weight.



1
P(3-HB)
$m \approx 9000$

2

More disturbing was the fact that both the chromatographic and the MS analyses indicated that the oligomer was composed of three major fractions (*i.e.*, it had a trimodal distribution).

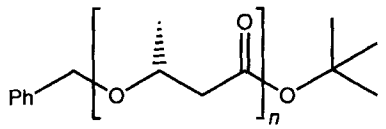
Some of us had speculated that the lack of complete degradation might have been due to the formation of a poly(enolate) and the presence of 'aggregations' in it and/or to the presence of 'substructures' in the P(3-HB) starting material [8]. Experiments, in which we tried to prove the intermediacy of enolates by attempted reactions with various electrophiles (deuteriolysis, addition to benzaldehyde, alkylation with benzyl bromide) and with P(3-HB) of various chain lengths (4, 20, 30, 60, and 10^4 3-HB units), were carried out in the meantime and were all completely unsuccessful. Therefore, we searched for reasons to explain the partial degradation and its trimodal pattern in the structure and properties of P(3-HB) and shorter oligomers of 3-HB. This involved the correlation of the results of our chemical degradations with the physical state of the polymer before and after degradation, and the use of nearly monodisperse oligomers of 3-HB to model the polymer.

2. Preparation and Analysis of Oligo(3-HB). – To determine the molecular weight of 3-HB oligomers, obtained in degradation experiments, without relying on questionable standards, we devised what we thought to be a systematic synthesis. We chose a benzyl-ether protection for the OH terminus and a *t*-butyl ester protection for the COOH terminus in an exponential segment-coupling strategy[3]) leading up to the production of the octamer [10]. Thus, two equimolar portions of the fully protected octamer **3a** were

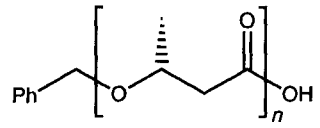
---

[2]) The same experiments were performed with *BIOPOL*, the copolymer of (*R*)-3-hydroxybutanoate and -pentanoate. Mass spectroscopic analysis indicated that the Et groups are distributed statistically on the polyester chain, replacing the Me groups randomly like in a *solid solution* [8].

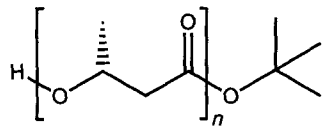
[3]) Using different protecting groups and a different coupling reagent, *Masamune* and coworkers have performed small-scale experiments leading to poorly characterized linear (up to 16 units) and cyclic (8 units) oligo(3-HB) derivatives [9].



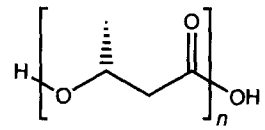
3a–7a



3b–7b



3c–7c



3d–7d

3 (n = 8)
4 (n = 16)
5 (n = 32)
6 (n = 64)
7 (n = 96)

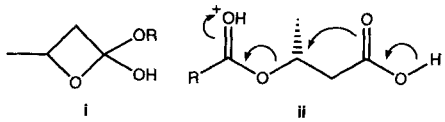
converted to the acid **3b** (CF_3CO_2H in CH_2Cl_2) and debenzylated to the hydroxy-ester **3c** (H_2/Pd-C in $HCONMe_2$), and the two components coupled after activation of the acid with oxalyl chloride. The resulting fully protected hexadecamer **4a** was 'dimerized' in the same way to give the 32mer **5a**, and there from the 64mer **6a** was prepared. Coupling of the 64mer **6b**, which had a free COOH group, with the HO-unprotected 32mer **5c** gave a P(3-HB) containing 96 units, at least that is what we thought we had in our hands! Up to the 32mer **5**, multigram quantities of the linear oligomers were prepared and purified by Et_2O extraction, fractional precipitation, and preparative size-exclusion chromatography (*Sephadex LH-60*). The synthesis was hampered by the fact that the oligo(3-HB) derivatives with larger chain lengths seem to hold back minute amounts of H_2O[4]). The relative stoichiometric amount of H_2O held back, with respect to the free functional groups, became larger as the chain lengths increased[5]). Most compounds **3**–**7** (**a**–**c**) were characterized by ^1H-, ^{13}C-NMR, and IR spectroscopy, by specific rotation, melting point, elemental analysis and by MS. The latter method revealed problems we had not been prepared for: with the 32mers three MS techniques could be applied, fast-atom-bombardment (LSI), plasma-desorption (PDI), and laser-desorption (MALDI) MS, and all three showed that single 3-HB units had been lost[6]). Thus, the 32mer **5a** contained varying amounts of 31- and 30mer (see *Fig. 1*). We found that this loss of 3-HB units occurs both

---

[4]) P(3-HB) is known to have H_2O uptake of 0.2% [11].

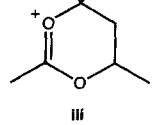
[5]) The moisture in the acid components **b** is removed during conversion of the acid to the acid chloride with excess $(COCl)_2$. The moisture in the alcohol component **c** is, of course, disastrous with respect to the coupling yield! For example, 0.2% by weight of H_2O in the 32mer **5c** (molecular weight 2828 Da) would destroy 30% of the acid chloride, when we form the 64mer **6a** in the corresponding coupling experiment. We have, therefore, used an 'after-coupling procedure', using 2,6-dichlorobenzoyl chloride after the reaction of the intermediate acid chloride with the long-chain alcohols (see *Exper. Part*).

[6]) The spectra clearly show that this loss is not caused by fragmentation in the mass spectrometer: the measured masses include the protecting groups on both ends of the chain. Reliable MS analysis of the high-molecular-weight oligomers became available to us only after we had finished the synthesis. The mechanism of loss of single units from the OH end may involve β-lactone (**i**) formation. For the cleavage of a 3-HB moiety from the COOH terminus, a β-lactone (**ii**) or a dioxinone (**iii**) intermediate may be involved. These possible mechanisms remind one of the term 'back-biting' used in polymer chemistry.



i

ii



iii

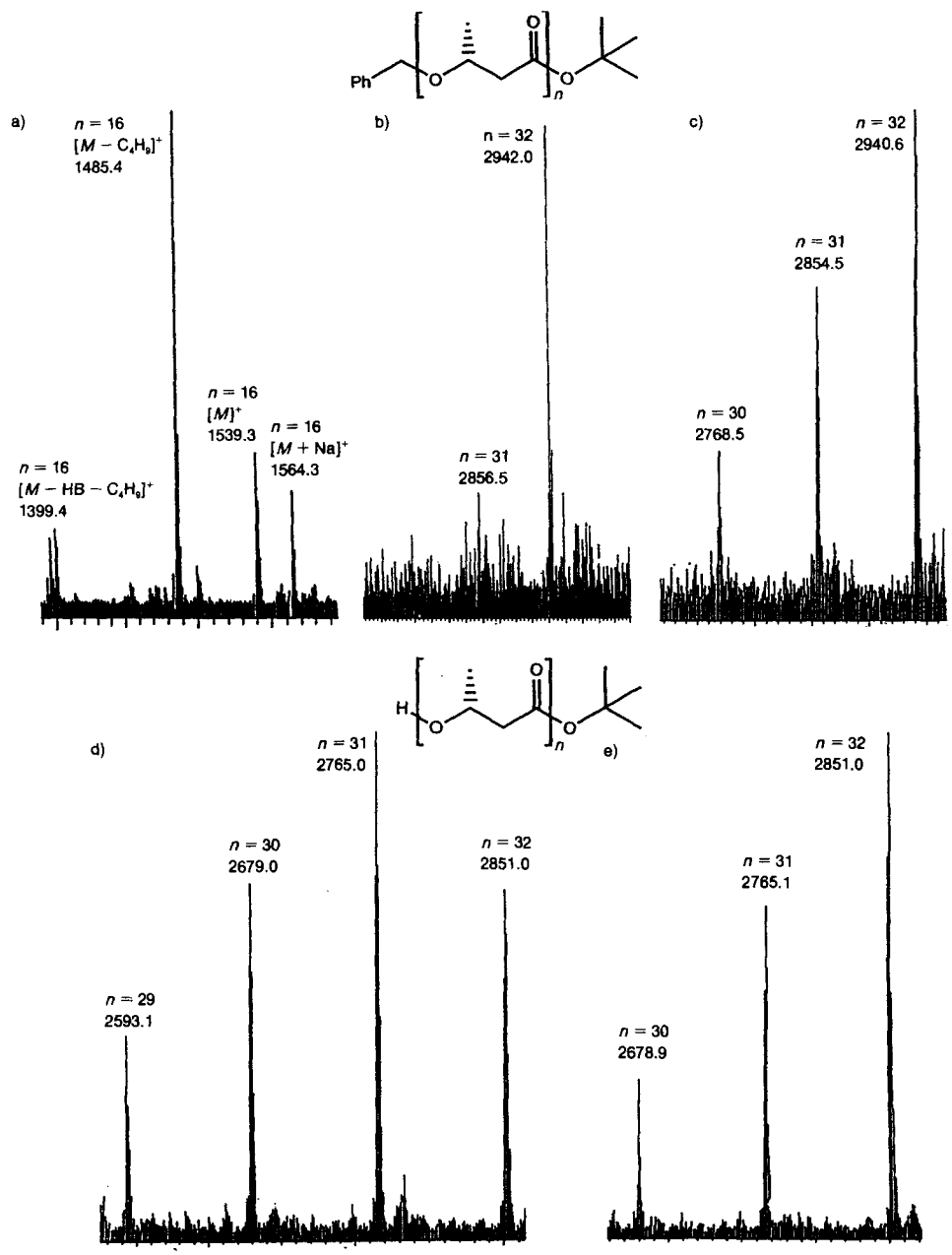
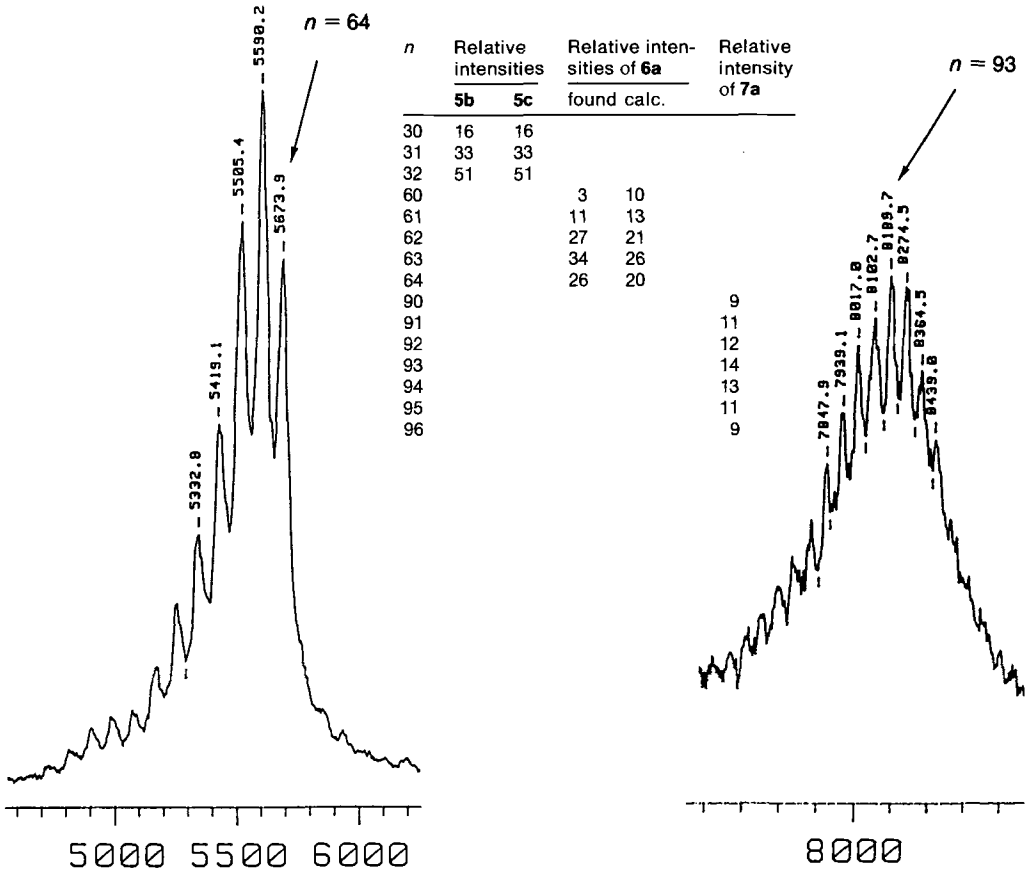


Fig. 1. *FAB Mass spectra of samples of the fully protected 16mer* **4a**, *the 32mer* **5a**, *and of the debenzylation product* **5c**. All spectra were obtained such that the measured ions had the (M + Na) masses, except the 16mer **4a** in part *a*. The signals at 1400 Da are due to fragmentation of the 16mer, because essentially no signals for the M^+ of the protected 15mer are visible. *a*) 16mer **4a**. *b*) and *c*) 32 mer **5a** obtained from two different runs, with the sample for *b prepared with* debenzylation of the 16mer **4a** in CF_3CH_2OH, for *c* in $HCONMe_2$ (DMF). *d*) Product of debenzylation of **5a** in DMF (70° to 20°), *e*) in CF_3CH_2OH. As can be seen by comparing *b* with *c*, and *d* with *e*, the loss of HB units occurs especially in DMF as a solvent and at elevated temperatures.

during debenzylation (**a** → **c**) and during *t*-butyl-ester cleavage (**a** → **b**), and that it occurs starting with the hexadecamer. While it can be avoided during debenzylation by the use of CF_3CH_2OH as solvent (*Fig. 1*), we have not found conditions, which would prevent the loss of 3-HB units, when cleaving the *t*-butyl-ester group. Trifluoroethanol and chlorinated hydrocarbons (CH_2Cl_2, $CHCl_3$, $ClCH_2CH_2Cl$) are the only solvents in which the higher oligomers, with and without protected end groups, are soluble at room temperature. This fact limits the synthetic methodology applicable for their conversions.

Since none of our purification methods could possibly separate *n*-mers from (*n* – 1)mers on a preparative scale, it was clear that the oligomers containing more than 32 3-HB units would consist of mixtures. The MALDI-MS method was elaborated for analysis of (3-HB)-oligomers (subject of a separate paper [12]), and used to determine the composition of the supposed 64- and 96mers **6a** and **7a** (see *Fig. 2*). Indeed, the main



n	Relative intensities		Relative intensities of **6a**		Relative intensity of **7a**
	5b	**5c**	found	calc.	
30	16	16			
31	33	33			
32	51	51			
60			3	10	
61			11	13	
62			27	21	
63			34	26	
64			26	20	
90					9
91					11
92					12
93					14
94					13
95					11
96					9

Fig. 2. *MALDI Mass spectra of the samples synthesized to be fully protected 64mer* **6a** *and 96mer* **7a**. As can be seen from *Fig. 1* and from the spectra shown here, the oligomer samples do not consist of uniform molecules, but are mixtures containing chains of less than the expected number of HB units as well. From the distribution of the 32mer **5b** used for assembling the 64mer **6a**, we have calculated the expected composition; the close resemblence of the calculated and measured distribution might be taken to indicate that hardly any further loss of HB units has occurred during the coupling step. The distribution of the sample meant to be the 96mer **7a** is also given; obviously, we have actually made a 93mer of $\bar{M}_w/\bar{M}_n \leq 1.001$!

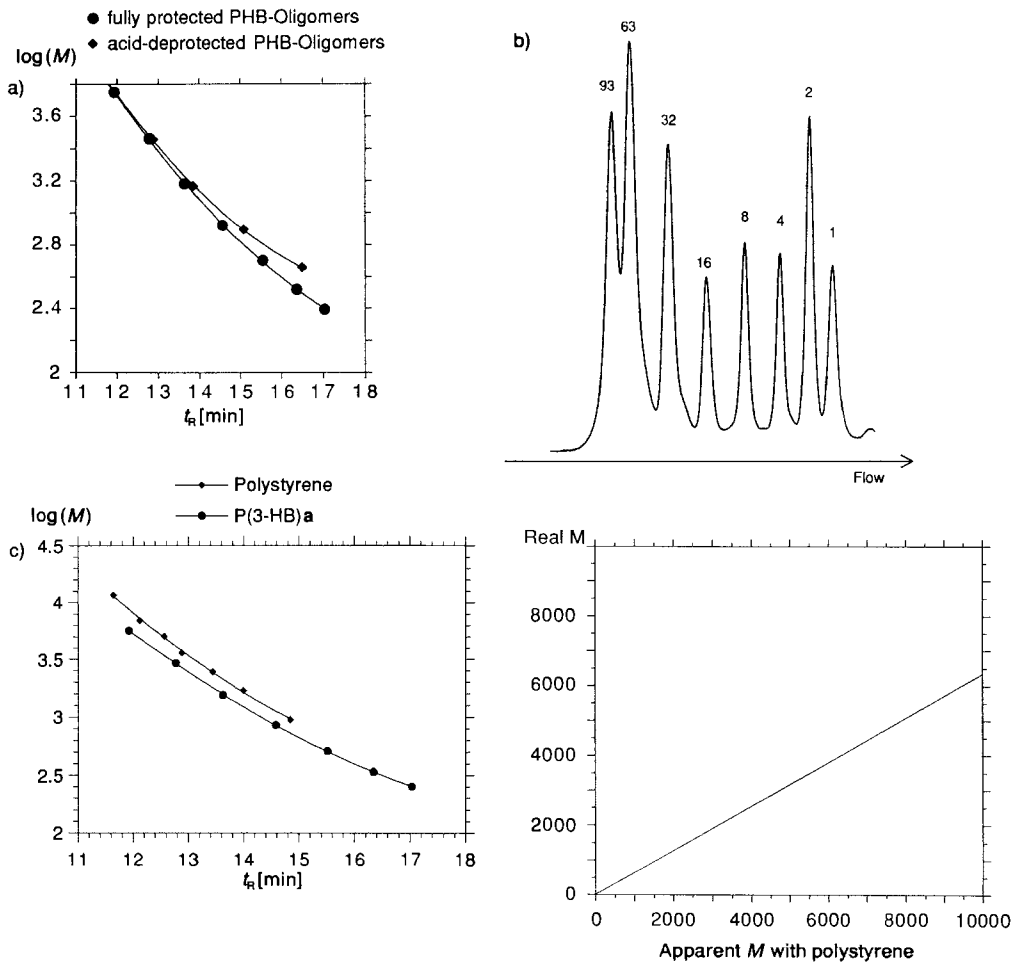
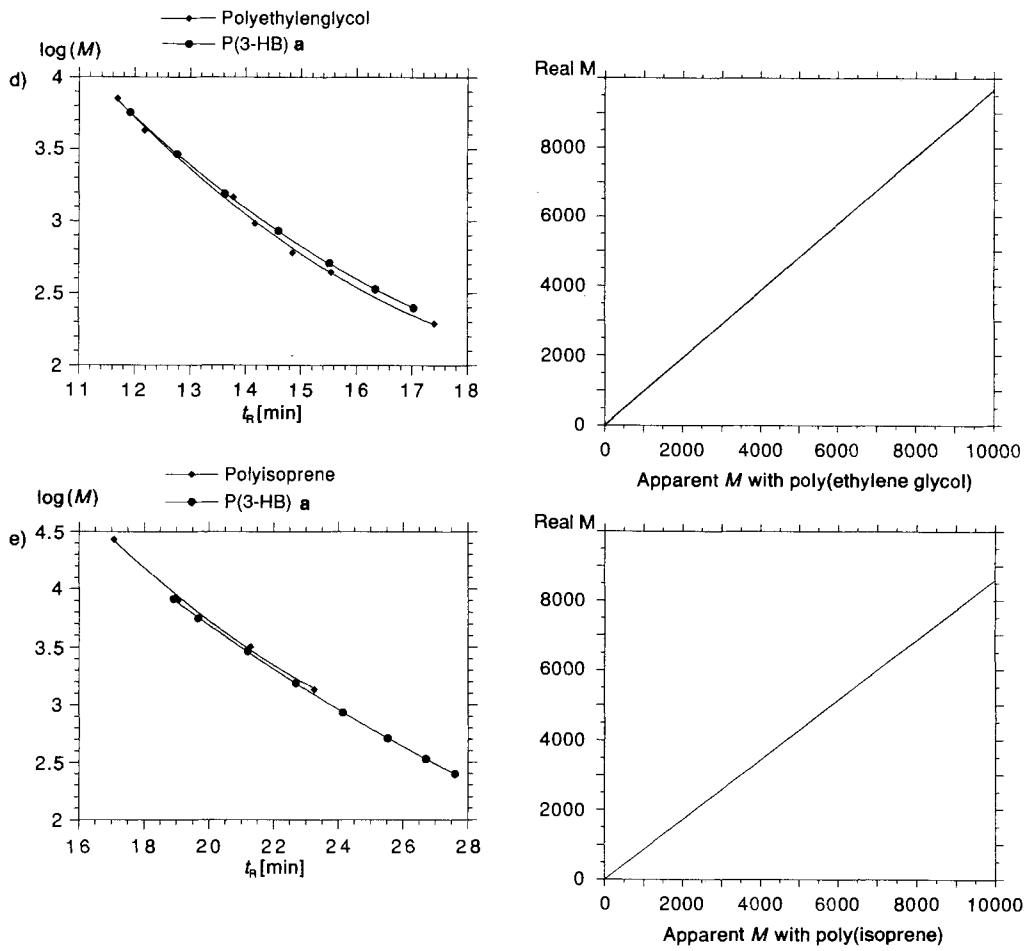


Fig. 3. *GPC Comparison of synthetic oligo(3-HB) derivatives* **3a**–**7a** *with standards of polystyrene (PS), poly(ethylene glycol) (PEG), and poly(isoprene) (PI).* The standards were purchased from *Polymer Laboratories Ltd.*, Essex Road,

components were found to be the 63mer and the 93mer, respectively. Calculation of the composition of the 63mer mixture, predicted from the composition of its precursors, may be considered as an indication that only a minor loss of 3-HB units could have happened during the coupling procedure[7]).

For GPC analyses, the samples of linear oligomers may be considered monodisperse ($M_w/M_n < 1.001$), so that while, our effort must be called utterly unsuccessful from the synthetic organic chemist's point of view, it is a full success with respect to the polymer

[7]) Further MS of oligomers described here have been shown in [3] [8] [12].



Church Stretton, Shropshire SY6 6AX, U.K. The diagram *a*, *c*, and *d* were obtained using two columns (*Shodex K 802* and *K 802.5*), the chromatogram *b* and the diagrams *e* using in addition a third column (*Shodex K 803*).

chemistry[8]). Mixtures of the synthesized oligomers were used to compare commercial polystyrene (PS), polyethylene glycol (PEG), and polyisoprene (PI) standards with P(3-HB) in $CHCl_3$ on size-exclusion columns (see *Fig. 3*). Correlation and comparison of the polymer retention times (t_R) show that the use of PS, PEG, and PI as standards for the analysis of P(3-HB) leads to molecular weights which are too high (by up to 50%) in the range of 5000–10000 Da (*Fig. 3*). Thus, previous determinations in this molecular-weight

[8]) Monodisperse oligomers of amino acids are well known, *e.g. Rothe* synthesized a poly(proline) of 40 units [13a]. Also monodisperse linear [13b] and branched [13c] paraffins containing up to 390 C-atoms were prepared, although these products were not analyzed using MS methods. An impressive example of a monodisperse high-molecular-weight compound is the dendrimer synthesized by *Xu* and *Moore*, which contains 1134(!) C-atoms [13d].

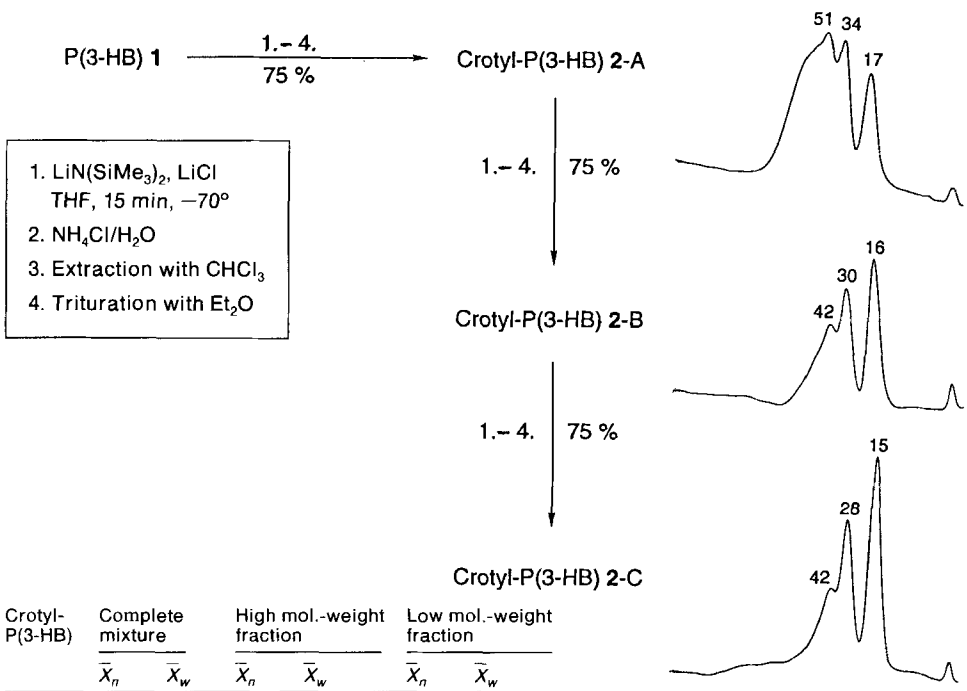
Table 1. *Comparison of the Molecular Weights of the Protected Synthetic Oligomers* **3a**–**7a** *with Those Obtained by Using Commercially Available Standards of Polystyrene (PS), Poly(ethylene glycol) (PEG), and Poly(isoprene) (PI), and the Calibration Functions Shown in* Fig. 3

Compound	Chain length	Calculated *M* [g/mol]	Apparent *M* [g/mol]		
			PS	PEG	PI
3a	8	853	1125	758	1033
4a	16	1542	2115	1441	1693
5a	32	2919	4119	2765	3095
6a	63	5588	8737	5650	6310
7a	93	8171	–	–	9273

range [14] must be corrected accordingly (see *Table 1*). It also transpires that the GPC retention times of oligo(3-HB) with full protection (**a** series) and with free acid end (**b** series) do not differ above the 32mer.

3. Degradation of P- and Oligo(3-HB) by $LiNR_2$ in Tetrahydrofuran at Dry-Ice Temperature. – We were now ready to go back to the degradation of P(3-HB) by Li-amide bases and to analyze the resulting mixtures properly. A sample of high-molecu-

Scheme. *Multiple Degradation of P(3-HB) by a Li Amide.* The GPC analysis was performed with the methyl esters. The series of three columns was calibrated with the synthetic oligo(3-HB) (*cf. Fig. 3*). Number- and weight-average degree of polymerizations were calculated for the complete mixture and also for the fractions above and below the trough, separating the lowest-molecular-weight peak from the rest of the chromatogram.



Crotyl-P(3-HB)	Complete mixture		High mol.-weight fraction		Low mol.-weight fraction	
	$\bar{X}_n$	$\bar{X}_w$	$\bar{X}_n$	$\bar{X}_w$	$\bar{X}_n$	$\bar{X}_w$
2-A	37	58	52	68	17	17
2-B	25	34	38	44	15	16
2-C	22	28	34	38	15	15

lar-weight P(3-HB), purified as described in [8], was suspended at room temperature in THF containing 3 equiv. of LiCl[9]) and stirred for 2 h. The mixture was then cooled to $-70°$ and combined, within 5 min, with 3 equiv. of $LiN(SiMe_3)_2$ (LHMDS)[10]) dissolved in the same solvent, whereupon the suspension containing visible flakes and flurries turned to a colorless, turbid, milky-looking liquid. After 15 min, the reaction mixture was quenched with NH_4Cl/H_2O at $-70°$ and worked up with $CHCl_3$. The dry crotyl-P(3-HB) **2**-A was triturated with Et_2O, filtered, dried again, and the material obtained was subjected to exactly the same procedure two more times, to give samples **2**-B and **2**-C (yield per step 65–80%)[11]) which were subjected to GPC as described in the previous section (see the *Scheme*). The mixtures were also analyzed by MALDI-MS, which nicely confirmed the molecular-weight distribution [12] obtained by the size-exclusion chromatography method. The three maxima of the molecular-weight distributions correspond to oligomers containing *ca.* 42, 28, and 15 3-HB units.

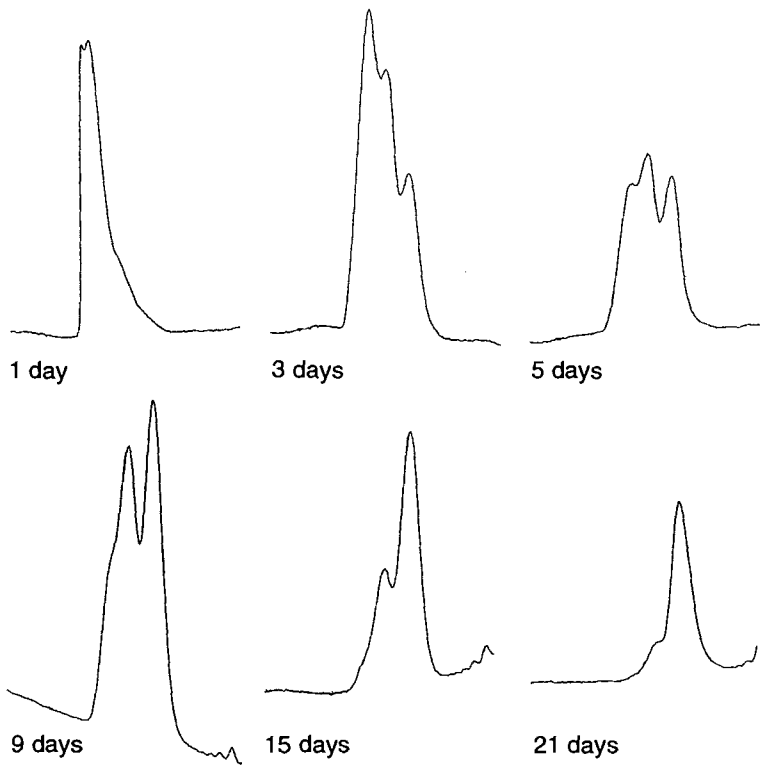


Fig. 4. *GPCs of degradation products of P(3-HB).* Molecular weight *ca.* 10^7, in boiling Et_3N. The shape of the curve obtained after the one-day treatment indicates that a lot of the P(3-HB) has still a higher molecular weight than the exclusion limit of the columns allows to detect.

---

[9]) Omission of LiCl does not significantly change the course of the reaction.

[10]) Another Li base used successfully is $LiN(CHMe_2)_2$. $LiNH_2$ and Ph_3CLi have not been successful under these conditions.

[11]) It has been found that P(3-HB) contains up to *ca.* 85% crystalline domains (see literature cited in [3]).

In another experiment, we heated a stirred suspension of P(3-HB), as supplied by *Imperial Chemical Industries*[12]), in neat Et_3N at reflux (b.p. 89°) for three weeks. GPC Analysis of samples withdrawn at different intervals again produced curves with three maxima (see *Fig. 4*).

In both cases, the chemical degradation reaction (base-induced β-elimination) was limited by a characteristic property of P(3-HB) which escaped the ETH team's knowledge for a long time [8]. In our previous degradation experiments for example, treatment of the P(3-HB) solution with acid [15] and its thermal degradation from the melt [16], our GPCs showed bell-shaped curves, rather than three peaks. We have now found the explanation for the tri-modal distribution, which is intrinsically connected with the solid-state structure of P(3-HB).

4. Solid-State Structures of P(3-HB) and Oligo(3-HB) Derivatives. – The crystallites formed when P(3-HB) is precipitated from dilute solution are in many ways typical of polymer crystals. They have a lamellar morphology (*i.e.*, they are thin platelet crystals) with the polymer chains running across the thin direction (the crystallographic *c*-axis) [17]. The length of the chains is very much greater than the crystal thickness, implying that a given chain must double back on itself and cross the crystal several times (see *Fig. 5* for a schematic representation of the molecular arrangement within polymer lamellae). The nature of this chain folding has been the subject of controversy and speculation over the last 30 years [18]. Points of discussion have been how tight the folds are, whether the chain re-enters the crystal at a lattice site adjacent to where it left, and whether molecules run across several crystals thereby tying them together.

In *Fig. 6*, we show a micrograph of isolated crystallites of P(3-HB) grown from dilute solution (0.01% *w/v* in propylene carbonate at 60°). These crystals were grown slowly and isothermally; in general, more rapid precipitation leads to multilayered crystallite aggregates.

Controlled degradation of carefully prepared, isolated polymer single crystals has been used to investigate the structure of the fold surface [19–21]. Etching away the loosest folds in the surface of the crystallites, using conditions under which the remaining segments are not soluble, has been used to examine the lengths of these segments. If there are tight folds (or 'adjacent re-entries'), degradation products with lengths equal to integer multiples of the lamellar thickness may result. In this way, *Welland et al.* [22] treated solid P(3-HB) with gaseous $MeNH_2$, and the analysis of the resulting oligo(3-HB) amides showed a bimodal distribution, just like the distribution we obtained from the products of the treatment of P(3-HB) with the very aggressive, strong base LHMDS. In our degradation, the P(3-HB) had not been carefully crystallized, but simply precipitated from a CH_2Cl_2 solution by $MeOH/H_2O$ [8], or obtained by evaporating a $CHCl_3$ solution to dryness (*Scheme*, and [8] [12]). In our case, not more than *ca.* 30% of the ester links in the P(3-HB) were cleaved.

With the samples from the LHMDS degradation and with the synthetic oligo(3-HB) **3a**–**7a** we were now in a position to confirm our speculation that we had encountered a

[12]) P(3-HB) of lot No. MBL 100/703 was obtained at that time from *Marlborough Biopolymers*, Billingham, now: *ZENECA Bio Products*, Biopolymers Group, PO Box 2, Billingham, Cleveland TS23 1 YN, U.K.

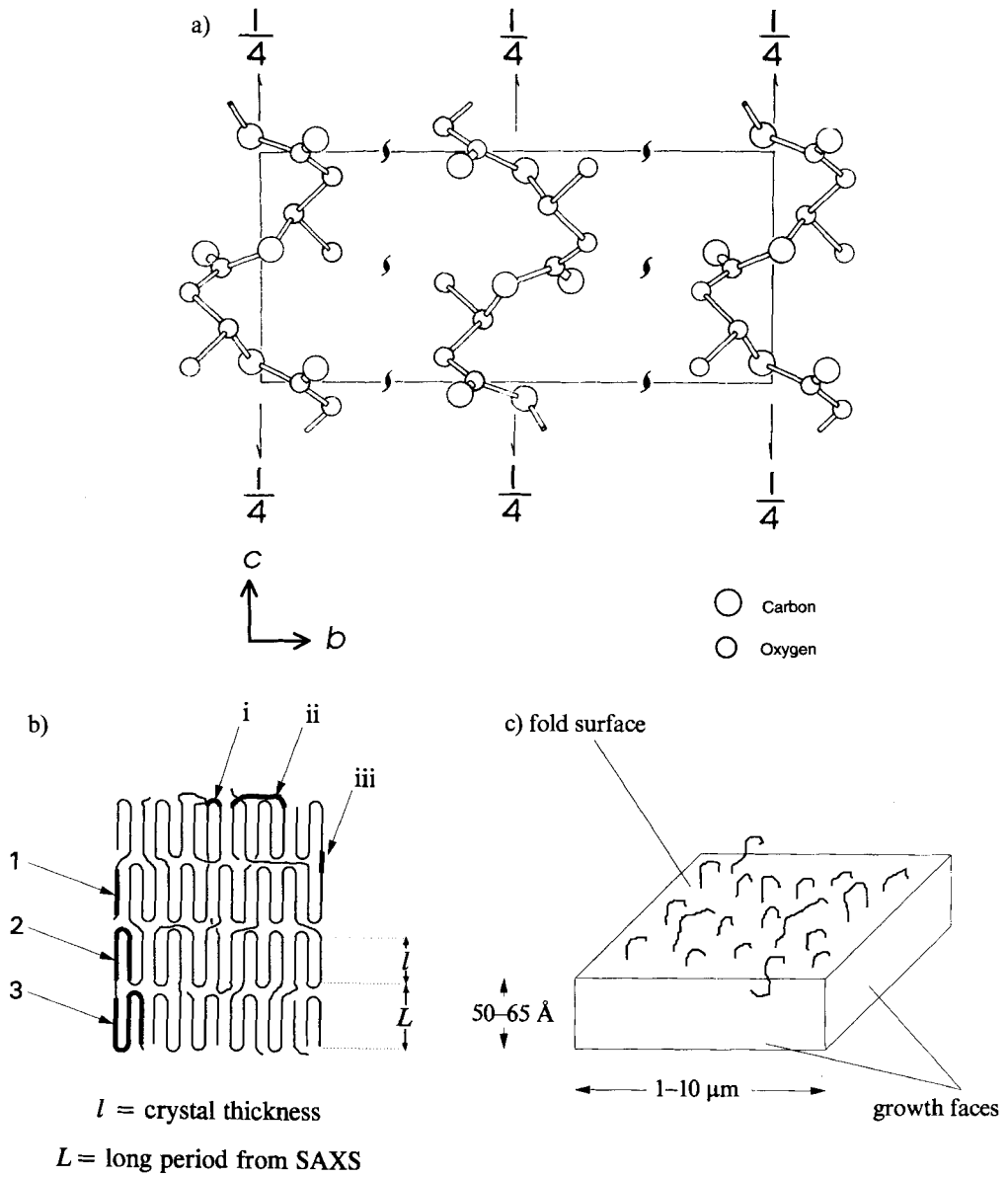


Fig. 5. *Structural model for chain-folded P(3-HB) crystals. a*) Crystal structure at an atomic scale. The S indicate a twofold screw axis. *b*) Schematic diagram showing a cross section through a stack of polymer lamellae. It illustrates some features of chain folding, including *i*) tight folds with adjacent re-entry, *ii*) loose folds with random re-entry, and *iii*) tie molecules connecting two or more crystals. l = crystallite thickness; L = long period ($= l +$ amorphous part) as determined by SAXS. The numbers 1, 2, and 3 indicate chain fragments with lengths in the ratio 1:2:3 that could remain after degradation, assuming that the etching agent can penetrate between the crystal layers. Note especially that the repeat period of a stack of crystals is slightly greater than the crystal core thickness, since the former includes space for folds and amorphous material between the crystals. *c*) A view of an isolated lamellar crystal indicating typical dimensions.

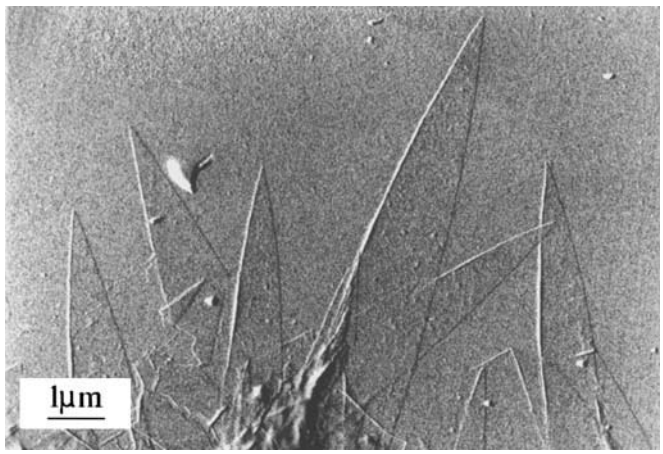


Fig. 6. *Lamellar crystallites of P(3-HB) grown from solution in propylene carbonate at 60°*

similar effect to that observed by *Welland et al.* by the use of transmission electron microscopy (TEM) and wide-angle X-ray scattering (WAXS).

The Bristol team studied the crystal structure and morphology of high-molecular-weight *s*-P(3-HB) which had been precipitated and dried immediately before Li-base treatment, and similar material immediately after it had been degraded for 15 min. The (nearly) monodisperse synthetic oligomers were also studied, both 'as received' and after recrystallization from dilute solution (0.01% *w/v* in propylene carbonate at 0°).

5. Results from Transmission Electron Microscopy and Small and Wide Angle X-Ray Scattering. – Results of these examinations are shown in *Figs. 7* and *8* and *Table 2*. The X-ray scattering results show that the undegraded P(3-HB) has the normal crystal structure and thickness. It was the observation that the long period (with allowance made for the uncertainty in the amount of amorphous material between the crystallites) gave a thickness of *ca.* 58 Å, which was similar to the length of the smallest oligomers seen after degradation (*ca.* 45 Å, measured by GPC), which first gave us the idea that the oligomers were stable for physical rather than chemical reasons. The TEM micrographs in *Fig. 8, d, e*, and *f*, show individual crystallites which are less regular, of smaller lateral dimensions and more aggregated than the 'ideal' crystals shown in *Fig. 6*. This is to be expected from a sample prepared by rapid precipitation rather than isothermal crystallization over the course of several days. After degradation the crystal structure, as shown by WAYS (*Fig. 7*), was unchanged, and the crystal thickness, as shown by SAXS (*Table 2*), was in the range of 58–47 Å. These results indicate that the crystal cores were relatively unaffected by the degradation.

The WAXS results show that the monodisperse synthetic oligomers have the same crystalline structures as the high-molecular-weight polymer. The presence or absence of protected end groups on the 16mer has no effect on the crystal structure, although it does seem to influence the way in which the crystals aggregate. The TEM shows that all the samples form lamellar crystals, with the 32mers being very similar to high-molecular-weight P(3-HB). It was especially interesting for us to see from the SAXS data (*Table 2*)

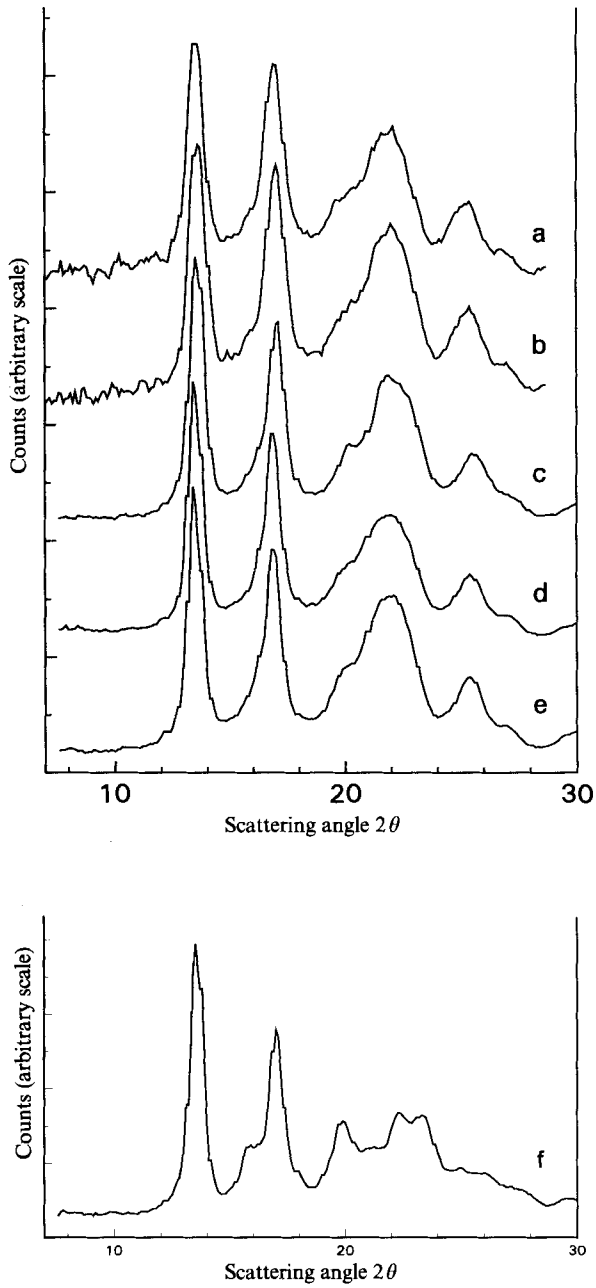


Fig. 7. *WAXS Profiles of* a) *P(3-HB)* (**1**), b) *bacterial P(3-HB) after degradation with Li base*, c) *the 16mer after removal of the protecting end groups* (**4d**), d) *the fully protected 16mer* (**4a**), e) *the fully protected 32mer of 3-HB* (**5a**). Apart from minor differences caused by changes in crystallinity and crystal perfection, all the patterns are the same. *f*) Distinctly different WAXS profile of 8mer **3d**.

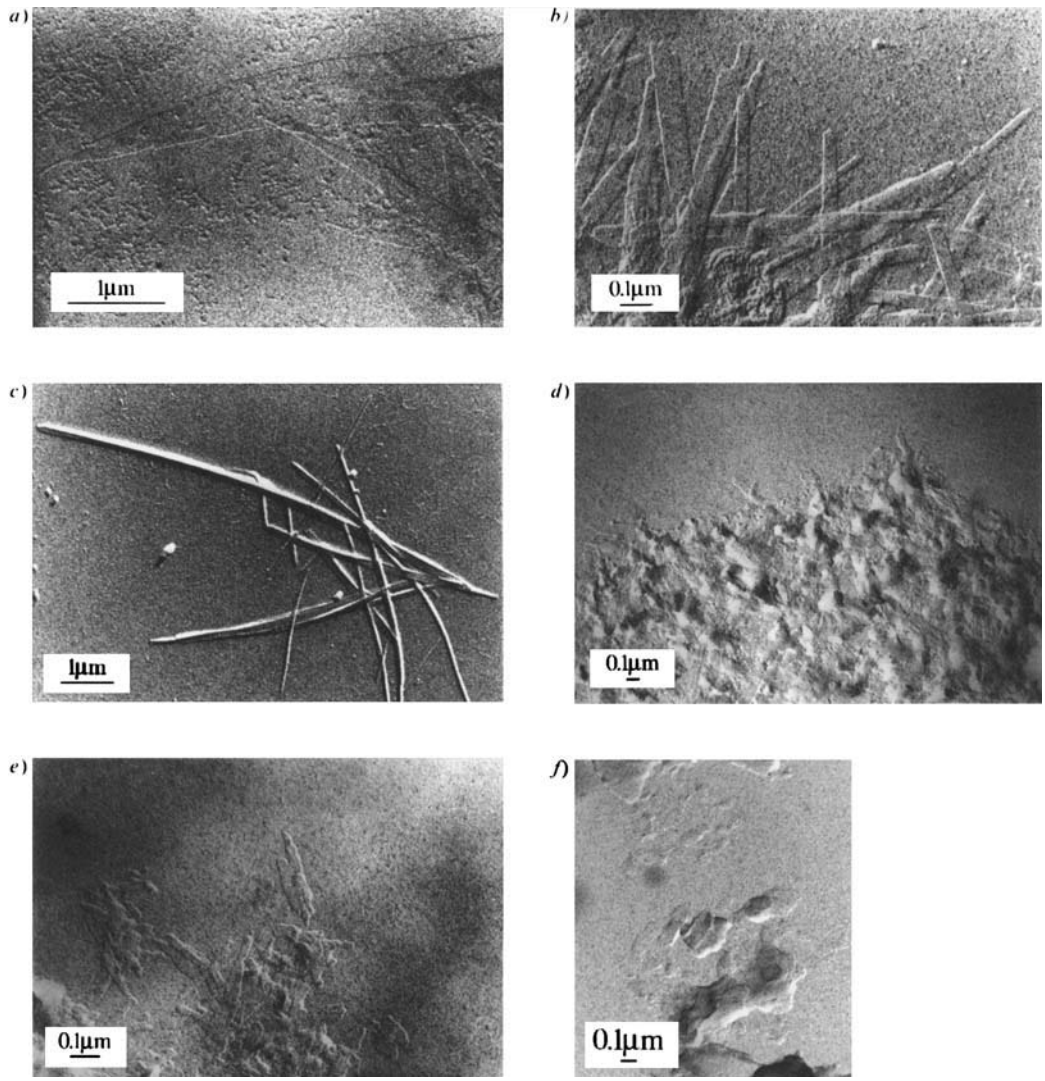


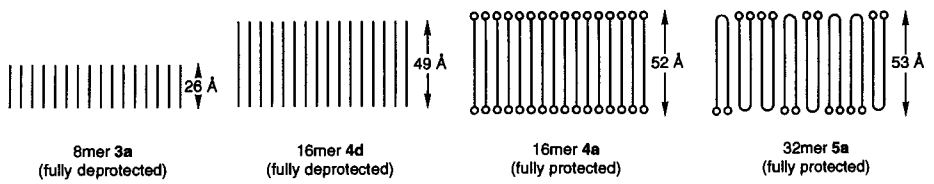
Fig. 8. *Transmission electron micrographs of lamellar crystals. a*) Protected 32mer **5a**, *b*) protected 16mer **4a**, *c*) unprotected 16mer (**4d**), *d*) aggregated P(3-HB) before base treatment, *e*) some isolated crystals of P(3-HB) found around the edge of an aggregate, *f*) remains of lamellae of P(3-HB) after base treatment. Samples for *a–c* were prepared by dissolving the oligomers in propylene carbonate (*ca.* 0.01% *w/v*) at 100° and then quenching to 0°. Samples for *e–f* were prepared by resuspending dried material in acetone.

that the crystal thickness of the 32mer (53 Å) was almost exactly equal to that of the 16mer (52 Å), which in turn corresponded closely to the length of 16 HB units in the P(3-HB) lattice (48 Å). This implies that the 32mer is chain-folded and, furthermore, that there is only a very short length of chain in the fold. Thus, we conclude that the P(3-HB) chain is capable of folding tightly with adjacent re-entry. The availability of

Table 2. *The Results of Small Angle X-Ray Scattering.*

The uncertainty of the crystallite thickness is ±2 Å. The number of monomers was calculated from the crystallite thickness with a helical pitch length of 5.96 Å for P(3-HB). Below the *Table*, we show an idealized presentation of the arrangements of the chains in the linear oligomers **3**, **4**, and **5** studied by SAXS. The unprotected 16mer **4d** forms crystallites which are slightly thinner than those of the *O*-benzylated *t*-butyl ester **4a**. The 32mer **5a**, having approximately the same lamellar thickness as the fully protected 16mer **4a**, must be present in a hair-pin arrangement, the turn of which cannot contain more than one or two HB units; otherwise, a smaller lamellar thickness should have been found. We do not know, whether the chains in **3a**, **4a**, and **4d** are arranged in an antiparallel fashion, known from PHB itself, or whether they are packed parallel, with all the acid or ester groups on one face and the alcohol or benzyl-ether moieties on the opposite face of the lamella.

Sample	Crystallite thickness [Å]	Number of monomers
Free 8mer **3d**	26	9
Protected 16mer **4a**	52	17
Free 16mer **4d**	49	16
Protected 32mer **5a**	53	18
Polymer **1**	65–50[a])	22–17
Degraded Polymer **2**-A[b])	54–40[a])	18–13



[a]) This represents the range between two extreme assumptions for interpreting the SAXS reflection: *i*) that the periodicity seen is the crystal thickness, *i.e.*, that there is no amorphous material between the lamellae; *ii*) that the volume fraction of crystal in the sample is 75%, and the amorphous material alternates with crystalline lamellae.

[b]) P(3-HB) (**1**) was degraded following the procedure outlined in the *Scheme*, omitting the extraction with $CHCl_3$ to preserve the lamellar structures.

monodisperse alkanes long enough to fold has been invaluable in understanding the behavior of chain-folded polyethylene [23], and we hope that the materials currently being synthesized in Zürich will extend this work to another polymer.

For our present purposes, we used our results on the oligomers to show that removal of the fold surface need not render P(3-HB) crystals unstable or would change the crystal structure. The observation of an unchanged crystal core after Li-base treatment is, therefore, consistent with the removal of amorphous chains which form the loose folds and inter-lamellar ties. The molecular trajectories which are most stable to degradation are tight folds and tight tie molecules. Inspection of *Fig. 5b* shows that this physical constraint naturally leads to chain fragments which have lengths in the ratio 1:2:3, where the unit corresponds to the lamellar thickness.

6. Conclusions and Speculations. – Degradation products **2** from the treatment of high-molecular-weight *s*-P(3-HB) **1** with Li amide base have a trimodal distribution of low molecular weights due to the crystalline structure of the polymer. The linear monodisperse oligomers, originally prepared for calibrating GPC columns for oligo(3-HB) molecular-weight determinations, can be used as models for the polymer, and can give some insights into the way of polyhydroxybutyrate chain folding.

Concerning the oligo(3-HB) occurring as a complex with calcium polyphosphate ($Ca^{2+} \cdot PP_i$) in cell membranes [7] [14] [24], the results described herein lead to the conclusion that the molecular weight of this *c*-P(3-HB) does not correspond to 120–200 [14] but rather to *ca.* 100–180 HB units.

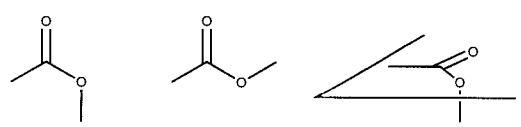
Reusch has proposed that *P*-helical $Ca^{2+} \cdot PP_i$ wrapped in a *P*-helix[13]) of *c*-P(3-HB) could act as an ion channel through a cell membrane or other hydrophobic barrier [14] [26]. In the proposed structure the P(3-HB) is required to form a helix of a pitch length of *ca.* 20 Å. It also forms unfavorable dihedral angles [10] along the oligo(3-HB) backbone. So far, we have not been able to suggest an alternative conformation of the polyester backbone fulfilling the following conditions: *a*) forming a helix wide enough to incorporate $Ca^{2+} \cdot PP_i$, *b*) having the C=O groups inside for Ca^{2+} complexation, *c*) turning the Me groups to the outside for exolipophilicity, and *d*) containing the favorable dihedral angles determined by X-ray crystal structure analysis of various oligolides from HB [10].

We can, on the other hand, model [10] the P(3-HB) 2_1-helix of *M*-helicity and a pitch length of *ca.* 6 Å, as derived independently by three groups [27] from fibre X-ray scattering measurements (see *Fig. 5, a*). Since P(3-HB) has repeatedly been found to form a helix of a pitch length of 6 Å, it is unlikely that a helix of a pitch length of 20 Å could also exist as a low-energy structure. Thus, we believe that, whatever structure the Ca^{2+} ion channel involving oligo(3-HB) has, it *must* contain a polyester chain with the stable dihedral angles[14]) listed in our previous paper [10]. The fact that oligo- and poly(3-HB) form crystallites of *ca.* 50-Å thickness, when crystallizing at temperatures below 40°, is intriguing when one considers that the sum of the lengths of two lipidic side chains of a phospholipid is also *ca.* 50 Å[15]). Thus, a chain of *ca.* 140 HB units in a 6-Å- pitch 2_1-helical conformation folded as in the lamellar crystallites could form 8 folds of the kind shown in *Fig. 5*, assuming tight folds of two HB units. The resulting structure would be a square-shaped sheet (*ca.* 50 × 50 × 5 Å) which can be viewed as a section cut out of the stretch fibre P(3-HB) arrangement, and, by the same token, out of a lamellar crystallite. In *Fig. 9*, we show a parallel projection of the P(3-HB) structure produced with the *MacMoMo* program [33] from published data [27a]. Neighboring helices running up and down parallel to the projection axis are shown outside or inside the frames. A stretch of eight

---

[13]) *M* (minus) and *P* (plus) nomenclature for assigning the sense of helicity, see [25].

[14]) In the modelling as published in [14] [26], ester groups have the unfavorable *sc*-conformation **iv** rather than the more stable *ap*-conformation **v** around the ester C–O bond. In our own modelling attempts, we even had to use conformations between *sc* and *ap* (resembling the transition state **vi** of rotation) in order to place all carbonyl O-atoms on the inside and the Me groups on the outside of the structure. In AcOMe, the *sc*-conformer is by *ca.* 4.3 kcal/mol higher in energy than the *ap*-conformer, and the barrier to rotation is *ca.* 10 kcal/mol. For conformational nomenclature, see [28]; for conformational energies, see textbooks (*e.g.* [29]).



iv **v** **vi**

[15]) The lipophilic part of trans-membrane proteins is typically in the range of 30 31 Å; however, if the helix is tilted with respect to the bilayer plane, a longer helix can be accommodated in the hydrophobic region [30] (see also the article of *Deisenhofer* and *Michel* concerning the membrane location of bacterio rhodopsin [31]). On the other hand, according to biochemistry textbooks [32], a phospholipid molecule has a length of *ca.* 35 Å, a typical phospholipid bilayer has a thickness of *ca.* 70 Å, a cell membrane of 60–100 Å.

antiparallel helices (the 50 × 50 Å sheet) is shown in red in *Fig. 9*. As can be seen, there are also eight consecutive antiparallel helices forming an ellipsoid arrangement (orange in *Fig. 9*). The mean of the long and short axes of this ellipse is *ca.* 20 Å. Thus, a 50-Å long cylinder of 20-Å diameter would result. This would be wide enough for housing a

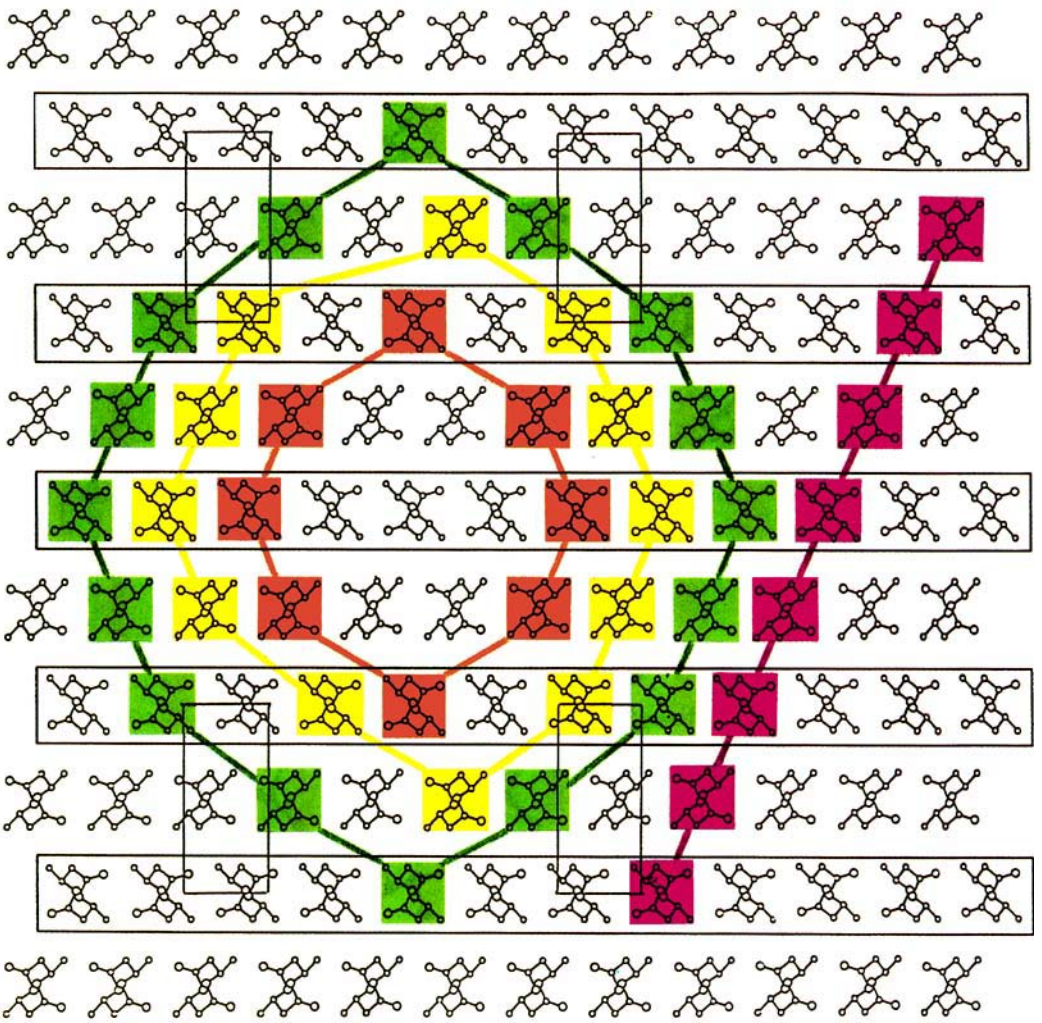


Fig. 9. *MacMoMo Presentation* [33] *of the structure of P(3-HB) as derived from fibre X-ray scattering and from molecular modeling* [27a]; *layers containing neighboring parallel and antiparallel* 2_1*-helices* (framed and non-framed); *a sheet and three tubules consisting of neighboring helices running alternatively up and down the lattice* (large circles represent O-, small circles C-atoms). The parallel helices running down from the carboxy to the hydroxy terminus are framed, those coming up un-framed. The carbonyl O-atoms are labelled black. The red group of helices marks a subsection which is part of a sheet-like arrangement (*cf. Fig. 5, a, b*, and *Table 2*). The orange, yellow, and green groups form tubules consisting of neighboring helices running up and down the lattice.

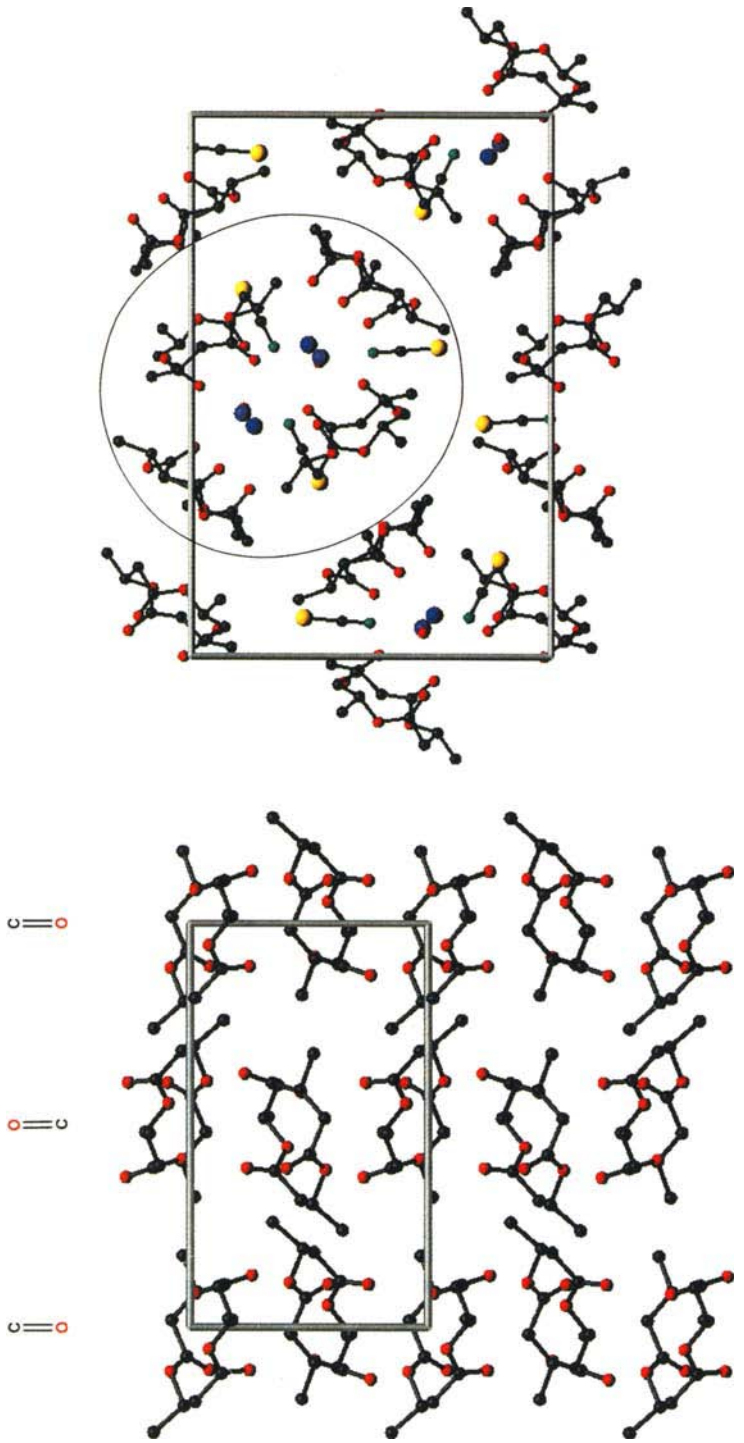


Fig. 10. *Sections from the crystal structures of the cyclic trimer of 3-HB, a triolide* (left), *and of the triolide complex with KSCN* (2 KSCN · 2 triolide · H_2O). The triolide molecules form sheets with the C=O groups aligned parallel in opposite directions on neighboring layers (*left*). In the complex, the triolide molecules rally round the K^+ ions, and formally a channel is formed with the K^+ ions inside, and the SCN^- N-atoms and triolide carbonyl O-atoms assembled along the inner wall. The outside of this channel may be considered lipophilic. For a discussion and comparison with similar complexes of NaSCN and $Ba(SCN)_2$, see [35].

$Ca^{2+} \cdot PP_i$ helix (*cf.* the *Reusch* proposal [14] [26]). On the other hand, a different set of oligo(3-HB) helices could easily be found (yellow and green in *Fig. 9*) which would provide a wider cylinder of 30- and 40-Å diameter, respectively) for adapting polyphosphates, such as DNA, with larger diameters. It should, however, be taken into consideration that, since the P(3-HB) chains would not be surrounded by a complete P(3-HB) crystal, but by lipid chains, the P(3-HB) chains would no longer be confined to the P(3-HB) lattice positions. Instead, the P(3-HB) chains would be free to adopt various positions within the lipid lattice, depending on the effect of the lipid chains upon them, and should be able to form cylinders of varying diameter. The suggestions delineated in *Fig. 9* are some of the possibilities.

The problem with this alternative proposal is *a*) that the resulting oligo(3-HB) $Ca^{2+} \cdot PP_i$ complex is not *exo*-lipophilic/*endo*-polarophilic to suit a lipidic membrane bilayer environment, and *b*) that not enough carbonyl O-atoms might be available on the inside of the tube to satisfy the Ca^{2+} ions' need for coordination[16]). We may speculate, however, that it is possible that within the lipid lattice the P(3-HB) helix becomes distorted[17]) and the C=O groups tend to lie inside the helix; thus satisfying the requirement *b*, having the C=O groups inside to allow for Ca^{2+} complexation.

In the presence of $Ca^{2+} \cdot PP_i$, other arrangements of the helices with formation of an oligo(3-HB) complex might be possible (compare the crystal packing of the triolide from 3-HB with that of the corresponding KSCN complex in *Fig. 10*). At present, we are trying to prepare, under a variety of conditions, samples of complexes from calcium oligophosphates and oligo(3-HB) suitable for solid-state structural investigations. We also plan to investigate the feasibility of our suggestion that the P(3-HB) chain could twist enough to force all the C=O groups to be on the inside of the helix. Hopefully, these investigations will lead to a more detailed picture of the Ca^{2+} channel through lipidic bilayers[18]) formed from the above-mentioned components.

We gratefully acknowledge the help of *R. Meier* in the preparation of some of the oligomers during his laboratory course in Organic Chemistry and *F. Kühnle* for his advice concerning structural details and for the preparation of the *MacMoMo* presentations. We thank *ZENECA Bio Products* (GB-Billingham) for generous supply of P(3-HB), the starting material for all our endeavors in this research project. We also thank the NMR service (PD Dr. *B. Jaun*, *B. Brandenberg*, *M. Sperl*), the MS service (Dr. *W. Amrein*, *R. Häfliger*, *O. Greter*), the microanalytical laboratory (*D. Manser*, *B. Sutter*), and the 'Kilolabor' (*K. Job*, *P. Kälin*) of the Laboratorium für Organische Chemie der ETH-Zürich. For the measurements of the MALDI-MS, we gratefully acknowledge the cooperation with the Corporate Analytical Research Department (Dr. *K.O. Börnsen*, Dr. *M. Schär*) of the *Ciba Ltd.* (CH–Basel). For financial support, we thank the ETH-Zürich (ETH project No. 0-20-007-90) and the *Sandoz AG* (CH–Basel). *Kathy Sykes* would like to thank the Science and Engineering Research Council for her quota award.

[16]) In complexes of amino acids, peptides, and related model systems, the Ca^{2+} shows six- to nine-fold coordination patterns. In Ca-binding proteins, the eightfold coordination pattern seems to be slightly preferred [34].

[17]) See the standard deviations of 5–18° in the corresponding angles in the oligolide crystal structures [10].

[18]) *Bramble* and *Reusch* have recently demonstrated [36] the formation of such Ca^{2+} channels by the bilayer clamp technique [37].

Experimental Part

General. All solvents were either *puriss. p. A.* quality or distilled over appropriate drying agents. M.p.: *Büchi 510*; not corrected. Specific rotation: *Perkin-Elmer 241* polarimeter. ^{1}H- and ^{13}C-NMR: *Bruker WH-300* or *Bruker AM-400*. All spectra were recorded using $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts are given in ppm relative to TMS and coupling constants are given in Hz. MS: LSI-MS: *VG-ZAB2-SEQ* with 3-nitrobenzyl alcohol. MALDI-MS (Matrix Assisted Laser Desorption and Ionisation MS): on a prototype (*LDI-1700*) instrument from *Linear Scientific, Inc.*, (Reno, NV, USA), using 2,5-dihydroxybenzoic acid as matrix. Elemental analysis: Microanalytical Laboratory of the ETH Zürich. GPC (Gel Permeation Chromatography): with the *Waters HPLC-GPC* system (*Waters 600E Multi Solvent Delivery System*). Detection was done with a *Waters 410 Differential Refractometer*. Chromatography was done using a series of two (*Shodex K-802, K-802.5*, stationary phase: styrene-divinylbenzene-copolymer) or three (additionally *Shodex K-803*) GPC columns with $CHCl_3$ (stabilized with amylene) as eluent, at 35°. Calibration was performed using the synthetic P(3-HB) derivatives of type **a** with defined molecular weights. Calculations ($\bar{M}_w$, $\bar{M}_n$, $\bar{X}_n$, and $\bar{M}_w/\bar{M}_n$) were done by numerical integration using a *Waters 746 Data Module* with *GPC-Package*. Polystyrene, polyethylene glycol and poly(1,4-isoprene) of narrow distributions were from *Polymer Laboratories Ltd.* (Essex Road, Church Stretton, Shropshire SY6 6AX, U.K.). Small Angle X-ray Scattering (SAXS) was performed using Ni filtered CuK_α radiation from a *Philips PW1729* generator running at 35 kV and 40 mA and a *Keisig* camera with pinhole collimation and specimen to film distance of 22 cm. Alternatively, we used Ni filtered CuK_α radiation from an *Eliot Marconi GX21* rotating anode generator running at 40 kV and 40 mA (fine focus) with a *Rigaku-Denki* camera with pinhole collimation and a specimen to film distance of 29 cm, and exposure time of 2 d. In both cases, the scattering was recorded on film and the long period of crystal stacks in the sample deduced from the radius of the SAXS reflection using *Braggs* law. Wide Angle X-ray Scattering (WAXS) was performed using graphite monochromated CuK_α from an *Eliot Marconi GX21* as described above, with collimation by two pairs of crossed slits. The patterns were recorded on a *Siemens* area detector with specimen to detector distance of 6 cm. The samples for transmission electron microscopy (TEM) were dispersed in a nonsolvent and deposited on carbon film supported by Cu grids. When the samples were dry they were shadowed with Pt/Pd to enhance the contrast and examined using a *Philips EM301* microscope operating at 80 kV.

Degradation of *s*-P(3-HB) with Base. – *α-(1-Oxobut-2-enyl)-ω-hydroxypoly{(R)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]}* (**2**). *Degradation with LiHMDS* [8]. A suspension of 3.0 g of reprecipitated P(3-HB) (34.8 mmol HB-units) and 3 equiv. of LiCl (4.43 g, 105 mmol) in 300 ml of THF (distilled over Na/benzophenone) was stirred for 2 h at r.t., then cooled to −74°. Lithium hexamethyldisilazanid (LiHMDS, 3 equiv.) generated from 22.5 ml (108 mmol) of hexamethyldisilazan in 10 ml of THF and 68 ml (109 mmol) of BuLi (1.6M in hexane), was added, as a cold suspension (−74°), to the P(3-HB) suspension during 10 min through *Teflon* tubing; the mixture was stirred at −70° for additional 10 min. A sat. aq. NH_4Cl soln. (30 ml) was added to the colorless, turbid mixture, which was then allowed to warm to r.t. After evaporation of the org. solvents, the aq. residue was extracted with $CHCl_3$ (3×). The combined org. layers were washed successively with sat. aq. $NaHCO_3$ (2×), 2N HCl, sat. aq. NaCl, and then dried ($MgSO_4$). After evaporation, Et_2O was added to the solid residue, and the suspension was stirred vigorously; the solid was filtered off and washed with Et_2O. Drying under high vacuum gave 2.24 g (79%) of a white powder **2**-A with a number average degree of polymerization of 32 (calculated from the ratio CH_3/CH_3O in the ^{1}H-NMR of a sample esterified with CH_2N_2). Treatment of 1.5 g of **2**-A under the same conditions yielded 1.19 g (79%) of a white powder **2**-B with a number average degree of polymerization of 21. 775 mg of the twice partially depolymerized **2**-B was again treated as described above and afforded 515 mg (66%) of a white powder **2**-C with a number average degree of polymerization of 20. ^{1}H-NMR (200 MHz): 7.02–6.85 (*m*, H–C(3) α-end group); 5.82–5.74 (*m*, H–C(2) α-end group); 5.32–5.16 (*m*, H–C(3)); 2.66–2.38 (*m*, CH_2(2)); 1.87–1.83 (*m*, CH_3(4) α-end group); 1.31–1.22 (*m*, CH_3(2)).

Degradation with Et_3N. P(3-HB) (2 g) was stirred under reflux (condenser sealed by a drying tube) in 40 ml of Et_3N. Samples were withdrawn after 1, 3, 5, 9, 15, and 21 d and dissolved in $CHCl_3$, washed with dil. HCl, dried ($MgSO_4$), and analyzed by anal. GPC. The chromatograms are shown in *Fig. 4*.

Synthesis of Linear Oligo(3-HB). – *General Procedure 1: Acid-Chloride Coupling Reaction.* The acid (**b** type) was dissolved in CH_2Cl_2, and 1.5 equiv. of oxalyl chloride $(COCl)_2$ were added. A few drops of DMF were added after 2 h, and the mixture was stirred for additional 2 h, until the gas evolution ceased. The solvent was removed and the solid residue dried in high vacuum. Under Ar, the acid chloride was dissolved in CH_2Cl_2 (dried over molecular sieves, 4 Å) and cooled to 0° in an ice bath. One equiv. of the alcohol and then 1.5 equiv. of pyridine (stored over 4-Å molecular sieves) were added in 5 min. For the 64mer **6** and the 96mer **7**, the solvents were further dried by direct filtration into the reaction flask over acidic alumina (activity 1). The extent of the reaction was

determined by anal. GPC, and was usually complete after stirring for 15 h at r.t. The mixture was diluted with CH_2Cl_2, washed with 1N HCl, sat. $NaHCO_3$, and sat. NaCl solns. Drying ($MgSO_4$) and evaporation yielded the crude products.

General Procedure 2: Removal of the t-*Butyl-Ester Protecting Group.* The benzyl-ether/*tert*-butyl-ester-protected oligomer (type **a**) was dissolved in CH_2Cl_2/CF_3COOH (volume ratio 5:1 to 1:1; dried over 4-Å molecular sieves) in a dried flask containing Ar as inert gas. Usually, the cleavage was complete (determined by ^{1}H-NMR) after 5 to 24 h at r.t. The solvent was removed *in vacuo* and the solid residue dried in high vacuum and used without further purification.

General Procedure 3: Removal of the Benzyl-Ether Protecting Group. A soln. of the benzyl-ether-protected oligomers (type **a** or **b**) in either CF_3CH_2OH or DMF was hydrogenated using 5–10% Pd-C (10%) as catalyst. After completion of the reaction (up to 8 h; determined by ^{1}H-NMR), 30 ml of CH_2Cl_2 were added, and the catalyst was removed by filtration using *Celite*. The solvent was removed *in vacuo* and the solid residue dried in high vacuum and used without further purification.

General Procedure 4: 'After-Coupling Procedure'. A mixture containing approximately equal amounts of *n*-meric acid (type **b**) and alcohol (type **c**) were dissolved in CH_2Cl_2 and pyridine. An excess of 2,6-dichlorobenzyl chloride in CH_2Cl_2 was added slowly and the reaction mixture stirred for an additional time. The extent of the reaction was determined by GPC. The products were isolated as described in *General Procedure 1*.

*α-Benzyl-ω-(*tert*-butoxy)hexadeca{(*R*)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]}* (**4a**). Following *General Procedure 1*, the acid chloride of **3b** was prepared using 9.32 g (11.7 mmol) of **3b** and 3.2 g (25 mmol) of $(COCl)_2$ in 40 ml of CH_2Cl_2. Coupling of this acid chloride (in 50 ml of CH_2Cl_2) with 9.10 g (11.7 mmol) of **3c** and 1.5 g (19 mmol) of pyridine in 25 ml of CH_2Cl_2 yielded 17.1 g of a white solid containing *ca.* 90% of **4a** (^{1}H-NMR and GPC). This crude product was refluxed in 250 ml of Et_2O for 12 h to dissolve unreacted starting material. The insoluble residue was treated again with 250 ml of Et_2O to yield 12.7 g of a white, insoluble solid containing less than 3% of the 8mer. M.p. 128.5–129.5°. $[\alpha]_D = -4.1$ ($c = 1.54$, CH_2Cl_2), $[\alpha]_{365} = 0.0$ ($c = 1.05$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1130*m*, 1100*m*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 7.35–7.20 (*m*, 5 arom. H); 5.35–5.20 (*m*, 15 H); 4.60–4.45 (*AB*, $J_{AB} = 11.6$, PhCH_2O); 4.05–3.95 (*m*, 1 H); 2.65–2.35 (*m*, 32 H); 1.45 (*s*, 9 H); 1.3–1.2 (*m*, 48 H). ^{1}H-NMR (100 MHz): 170.50; 169.32; 169.23; 169.14; 138.56; 128.33; 127.63; 127.52; 80.91; 71.95; 70.81; 67.99; 67.62; 67.58; 67.35; 42.13; 42.01; 40.91; 40.87; 40.82; 28.06; 19.86; 19.81; 19.78. LSI-MS: 1673.2 (1.4, $[M + Cs]^+$), 1563 (< 1, $[M + Na]^+$), 1540 (< 1, M^+), 689 (2.6), 603 (3.0), 517 (2.2), 431 (4.1), 345.3 (7.4), 259 (11), 173.3 (20), 155 (65), 91 (33), 87 (28), 79 (21), 69 (100). Anal. calc. for $C_{75}H_{112}O_{33}$: C 58.43, H 7.32; found: C 58.09, H 7.34.

To increase the yield of **4a**, the combined Et_2O-soluble extracts were dried in high vacuum, and the light brown product (4.0 g) was dissolved in 15 ml of CH_2Cl_2 and 1.2 ml of pyridine. 2,6-Dichlorobenzoyl chloride (1.0 g, 4.8 mmol) in 5 ml of CH_2Cl_2 was added, as described in *General Procedure 4*. This crude product contained *ca.* 65% **4a** which was purified as outlined above to yield 2.6 g of a white solid containing less then 5% of the 8mer (GPC).

*α-Benzyl-ω-hydroxyhexadeca{(*R*)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]}* (**4b**). Following *General Procedure 2*, 5.50 g (3.57 mmol) of **4a** in 30 ml of CH_2Cl_2 were treated with 10 ml of CF_3COOH. The reaction was complete after 3 h at r.t. After the addition of 20 ml of 1,2-dichloroethane, the solvents were removed *in vacuo*. The crude residue was dissolved in CH_2Cl_2, the solvent stripped off to remove traces of CF_3COOH. This was repeated two more times, then the residue was dried for 8 h in high vacuum (50°/0.05 Torr) to give 5.26 g (99%) of a yellowish solid which was pure according to the ^{1}H-NMR. M.p. 122.0–122.5° $[\alpha]_D = -4.8$ ($c = 1.38$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1130*m*, 1100*w*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 7.35–7.20 (*m*, 5 arom. H); 5.35–5.20 (*m*, 15 H); 4.55–4.45 (*AB*, $J_{AB} = 11.6$, PhCH_2O); 4.05–3.95 (*m*, 1 H); 2.65–2.35 (*m*, 32 H); 1.3–1.2 (*m*, 48 H). ^{13}C-NMR (100 MHz): 172.33; 170.52; 169.67; 169.36; 169.34; 169.30; 169.24; 169.21; 169.17; 169.09; 138.52; 128.33; 127.65; 127.52; 71.94; 70.79; 68.06; 67.75; 67.69; 67.63; 67.58; 67.36; 42.11; 40.88; 40.80; 40.35; 19.92; 19.84; 19.76; 19.66; 19.51. LSIMS: 1507.5 (68, $[M + Na]^+$), 1421.5 (6.4, $[M + Na - 86]^+$), 1417.5 (5.4), 431 (2.5), 345.3 (3.9), 259 (5.8), 241 (10), 173.3 (16), 155 (100), 91 (41), 87 (20), 77 (17), 69 (60). Anal. calc. for $C_{75}H_{112}O_{33}$: C 58.43, H 7.32; found: C 58.09, H 7.34.

α-Hydro-ω-(tert-*butoxy)hexadeca{(*R*)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]}* (**4c**). Compound **4a** (5.50 g, 3.57 mmol) and 0.8 g of Pd-C in 200 ml of DMF were hydrogenated at 50° as described in *General Procedure 3*. Drying in high vacuum (0.05 Torr) at 50–60° yielded 5.10 g (98%) of a slightly yellowish solid which was essentially pure. M.p. 130–131°. $[\alpha]_D = -4.2$ ($c = 1.00$, CH_2Cl_2), $[\alpha]_{365} = 0.0$ ($c = 1.00$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1130*m*, 1100*m*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 7.35–7.20 (*m*, 5 arom. H); 5.40–5.20 (*m*, 15 H); 4.25–4.10 (*m*, 1 H); 3.09 (*d*, $J = 3.9$, OH); 2.65–2.35 (*m*, 32 H); 1.45 (*s*, 9 H); 1.35–1.15 (*m*, 45 H); 1.22 (*d*, $J = 6.3$, 3 H). ^{13}C-NMR (100 MHz): 172.00; 169.40; 169.31; 169.18; 169.13; 80.90; 67.98; 67.75;

67.62; 67.53; 64.38; 43.25; 41.99; 40.80; 28.03; 22.52; 19.88; 19.76. LSI-MS: 1601.8 (< 1, $[M + Cs]^+$), 1473.8 (< 1, $[M + Na]^+$), 1395.7 (< 1, $[M - 57 + H]^+$), 1387.7 (≪ 1, $[M - 86 + Na]^+$), 689.4 (< 1), 603.4 (1), 517.3 (1), 431.3 (2), 345.3 (4), 259.2 (6), 241 (11), 173.3 (14), 155 (56), 87 (25), 69 (100).

α-Hydro-ω-hydroxyhexadeca{(R)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]} (**4d**). Following *General Procedure 3*, the benzyl-ether group of 0.5 g (0.34 mmol) **4b** was removed using 0.05 g Pd-C in 30 ml of trifluoroethanol in 5 h at r.t. After drying for 12 h in high vacuum (0.05 Torr) at 50°, 461 mg (98%) of a white solid were obtained, which were reprecipitated twice from CH_2Cl_2/pentane at −30°. An anal. pure sample for the spectroscopic data was obtained by GPC on *Sephadex LH 20* in CH_2Cl_2. M.p. 134.0–134.5. $[\alpha]_D = -7.9$ ($c = 1.29$, CH_2Cl_2). IR (KBr): 3470*w*, 2990*w*, 2970*w*, 3700–2500*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*vs*, 1230*s*, 1185*m*, 1130*s*, 1100*s*, 1060*s*, 980*m*. ^{1}H-NMR (400 MHz): 5.40–5.20 (*m*, 15 H); 4.35–4.15 (*m*, 1 H); 2.70–2.40 (*m*, 32 H); 1.35–1.20 (*m*, 45 H); 1.22 (*d*, $J = 6.3$, 3 H). ^{13}C-NMR (100 MHz): 171.96; 169.64; 169.39; 169.35; 169.32; 169.26; 169.19; 169.14; 169.07; 68.01; 67.71; 67.65; 67.59; 67.51; 64.36; 43.18; 40.84; 40.75; 40.54; 22.27; 19.90; 19.83; 19.71; 19.62. LSIMS: 1416 (10, $[M + Na]^+$), 328.7 (6), 154.8 (45), 69 (100). Anal. calc. for $C_{64}H_{98}O_{33}$: C 55.09, H 7.08; found: C 54.74, H 7.22.

*α-Benzyl-ω-(*tert*-butoxy)dotriaconta{(R)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]}* (**5a**). Following *General Procedure 1*, the acid chloride of **4b** was prepared using 5.2 g (3.50 mmol) of **4b** and 2.2 g (18 mmol) of $(COCl)_2$ in 13 ml of CH_2Cl_2. Coupling of this acid chloride (in 18 ml CH_2Cl_2) with 5.08 g (3.50 mmol) of **4c** and 1.0 g (13 mmol) of pyridine yielded 9.73 g of a white solid containing *ca.* 60% of **5a** (GPC). This crude product was purified by precipitation from DMF: 8.7 g were dissolved in 100 ml DMF at 50° and slowly cooled to r.t. The precipitate was pelleted by centrifugation and the yellow supernatand removed. This procedure was repeated twice to yield 4.56 g of **5a** containing less than 5% 16mer. To increase the yield of **5a**, the yellow solid obtained by evaporation of the DMF solns. was dissolved in 10 ml of CH_2Cl_2, and 1 ml of pyridine and then 0.61 g (2.9 mmol) of 2,6-dichlorobenzoyl chloride in 5 ml of CH_2Cl_2 were added during 3 h and stirred for an additional 2 h, as described in *General Procedure 4*. The crude product contained *ca.* 65% **5a** which was purified as described above to yield 2.1 g of a yellowish solid containing *ca.* 5% of the 16mer (GPC). The anal. data were determined from 0.5 g of this product purified by GPC on *Sephadex LH 60* in CH_2Cl_2. M.p. 154.0–154.5°. $[\alpha]_{365} = +4.7$ ($c = 1.41$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1185*m*, 1135*m*, 1100*m*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 7.35–7.20 (*m*, 5 arom. H); 5.30–5.20 (*m*, 31 H); 4.60–4.45 (*AB*, $J_{AB} = 11.6$, PhCH_2O); 4.05–3.95 (*m*, 1 H); 2.65–2.35 (*m*, 64 H); 1.45 (*s*, 9 H); 1.28 (*d*, $J = 6.3$, 96 H). ^{13}C-NMR (100 MHz): 170.49; 169.32; 169.23; 169.13; 138.54; 128.32; 127.62; 127.51; 71.94; 70.80; 67.98; 67.61; 67.34; 42.12; 42.01; 40.90; 40.81; 28.04; 19.85; 19.77. LSIMS: 2940.6 (100, $[M + Na]^+$), 2854.5 (64, $[M - 86 + Na]^+$), 2768.5 (32, $[M - 172 + Na]^+$). MALDI-MS: 2945.9 (100, $[M + Na + 5]^+$), 2854.5 (64, $[M - 86 + Na]^+$), 2768.5 (23, $[M - 172 + Na]^+$). PDI-MS: 2941.1 (100, $[M + Na]^+$), 2855.2 (83, $[M - 86 + Na]^+$), 2770.0 (29, $[M - 172 + Na]^+$). Anal. calc. for $C_{139}H_{208}O_{65}$: C 57.19, H 7.18; found: C 57.14, H 7.27.

α-Benzyl-ω-hydroxydotriaconta{(R)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]} (**5b**). Following *General Procedure 2*, 2.0 g (0.69 mmol) of **5a** in 10 ml of CH_2Cl_2 were treated with 5 ml of CF_3COOH. The reaction was complete after 5 h at r.t. After the addition of 5 ml of 1,2-dichloroethane, the solvents were removed *in vacuo*, and the residue was dried for 0.5 h in high vacuum (50°/0.05 Torr). To remove trace amounts of CF_3COOH, the crude product was dissolved in CH_2Cl_2 and washed successively with sat. aq. $NaHCO_3$ (2×), 1N HCl, and brine. Removal of the solvent *in vacuo* and drying in high vacuum (50°/0.05 Torr) for 12 h yielded 1.99 g (99%) of a yellowish solid which was essentially pure (^{1}H-NMR). M.p. 154.5–155.0°. $[\alpha]_{365} = +4.2$ ($c = 1.32$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1130*m*, 1100*w*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 7.35–7.20 (*m*, 5 arom. H); 5.35–5.20 (*m*, 31 H); 4.55–4.45 (*AB*, $J_{AB} = 11.6$, PhCH_2O); 4.05–3.95 (*m*, 1 H); 2.65–2.35 (*m*, 64 H); 1.28 (*d*, $J = 6.3$, 96 H). ^{13}C-NMR (100 MHz): 169.79; 169.41; 169.34; 169.16; 169.07; 128.33; 127.63; 127.52; 71.94; 70.61; 68.17; 67.79; 67.72; 67.63; 67.36; 42.13; 40.92; 40.82; 19.99; 19.86; 19.77; 19.66. LSI-MS: 2885.8 (100, $[M + Na]^+$), 2799.6 (64, $[M - 86 + Na]^+$), 2713.6 (32, $[M - 172 + Na]^+$).

*α-Hydro-ω-(*tert*-butoxy)dotriaconta{(R)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]}* (**5c**). Following *General Procedure 3*, 0.25 g of Pd-C were added to 2.0 g (0.69 mmol) of **5a** dissolved in 200 ml of DMF at 60°, and hydrogenated, while the mixture was allowed to cool to r.t. After 8 h, this procedure was repeated. Evaporation of the solvent and drying in high vacuum (50°/0.05 Torr) yielded 1.98 g (98%) of a brownish solid which was essentially pure (^{1}H-NMR). M.p. 154–155°. $[\alpha]_{365} = +4.4$ ($c = 1.43$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1130*m*, 1100*m*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 7.35–7.20 (*m*, 5 arom. H); 5.40–5.20 (*m*, 31 H); 4.25–4.10 (*m*, 1 H); 3.09 (*d*, $J = 3.9$, OH); 2.65–2.35 (*m*, 64 H); 1.45 (*s*, 9 H); 1.28 (*d*, $J = 6.3$, 93 H); 1.22 (*d*, $J = 6.3$, 3 H). ^{13}C-NMR (100 MHz): 172.00; 169.43; 169.34; 169.15; 68.10; 68.00; 67.86; 67.76; 67.63; 67.55; 64.39; 43.26; 42.00; 40.81; 28.05; 22.54; 19.89; 19.77. LSI-MS: 2851.0 (100, $[M + Na]^+$), 2765.1 (64, $[M - 86 + Na]^+$), 2678.9 (32, $[M - 172 + Na]^+$).

α-Hydro-ω-hydroxydotriaconta{(R)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]} (**5d**). Following *General Procedure 2*, 1.0 g (0.34 mmol) of **5a** was deprotected using 30 ml of CH_2Cl_2 and 10 ml of CF_3COOH. After removal of the solvent and drying in high vacuum (0.05 Torr, 50°) for 12 h, the yellowish solid was hydrogenated in 50 ml of CF_3CH_2OH with 0.1 g of Pd-C over 5 h at r.t. (*General Procedure 3*). Drying of the product for 18 h in high vacuum (50°/0.05 Torr) yielded 0.84 g (89%) **5d**. The anal. data were determined from 0.5 g of this product purified by GPC on *Sephadex LH 60* in CH_2Cl_2. M.p. 154.0–154.5°. $[\alpha]_{365} = +3.5$ ($c = 1.09$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1130*m*, 1100*m*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 5.40–5.20 (*m*, 31 H); 4.35–4.15 (*m*, 1 H); 2.70–2.40 (*m*, 64 H); 1.28 (*d*, $J = 6.3$, 93 H); 1.22 (*d*, $J = 6.3$, 3 H). ^{13}C-NMR (100 MHz): 172.00; 169.77; 169.38; 169.33; 169.16; 169.07; 68.15; 67.76; 67.72; 67.63; 67.55; 64.41; 43.26; 40.92; 40.82; 40.51; 40.36; 22.54; 19.98; 19.89; 19.78; 19.66. LSI-MS: 2795 (96, $[M + Na]^+$), 2709 (100, $[M - 86 + Na]^+$), 2622.9 (42, $[M - 172 + Na]^+$). MALDI-MS: 2772.8 (97, $[M + H]^+$), 2687 (100, $[M - 86 + H]^+$), 2601.2 (51, $[M - 172 + H]^+$). Anal. calc. for $C_{128}H_{194}O_{65}$: C 55.44, H 7.05; found: C 55.22, H 7.20.

α-Benzyl-ω-(tert-butoxy)tetrahexaconta{(R)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]} (**6a**). Following *General Procedure 1*, the acid chloride of **5b** was prepared using 1.90 g (0.066 mmol) of **5b** and 2.2 g (17 mmol) of $(COCl)_2$ in 10 ml of CH_2Cl_2. Coupling of this acid chloride (in 10 ml CH_2Cl_2) with 1.90 g (0.67 mmol) of **5c** and 1.0 g (13 mmol) of pyridine yielded 3.75 g of a white solid containing *ca.* 45% of **6a** (GPC). This crude product was purified by precipitation from DMF: 8.7 g were dissolved in 50 ml of DMF at 50° and slowly cooled to r.t. The precipitate was pelleted by centrifugation and the yellow supernatand removed. This procedure was repeated twice to yield 0.64 g of **6a** containing less than 8% of 32mer. To increase the yield of **6a** the yellow solid obtained by evaporation of the DMF solns. was dissolved in 10 ml of CH_2Cl_2 and 1 ml of pyridine, and then 4.4 g (21 mmol) of 2,6-dichlorobenzoyl chloride in 10 ml of CH_2Cl_2 were added during 5 h and stirred for additional 2 h, as described in *General Procedure 4*. The crude product contained *ca.* 60% **6a** which was purified as described above to yield 1.84 g of a yellowish solid. 1.5 g of this product were further purified by GPC (*Sephadex LH 60*, CH_2Cl_2) and were then used for the synthesis of the 96mers. M.p. 153.5–154.5°. $[\alpha]_{365} = +7.9$ ($c = 0.96$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1135*m*, 1100*m*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 7.35–7.20 (*m*, 5 arom. H); 5.30–5.20 (*m*, 63 H); 4.60–4.45 (*AB*, $J_{AB} = 11.6$, PhCH_2O); 4.05–3.95 (*m*, 1 H); $\nu_A = 2.61$, $\nu_B = 2.48$ (*AB* of *ABX*, $J_{AX} = 7.4$, $J_{BX} = 5.8$, $J_{AB} = 15.5$, 128 H); 1.45 (*s*, 9 H); 1.28 (*d*, $J = 6.3$, 192 H). ^{13}C-NMR (100 MHz): 169.13; 128.32; 127.62; 127.51; 71.93; 70.80; 67.98; 67.61; 67.41; 67.34; 42.12; 41.99; 40.80; 28.04; 19.85; 19.77. MALDI-MS: 5674 (77, $[M - 3]^+$), 5590 (100, $[M - 87]^+$), 5505 (81, $[M - 172]^+$), 5419 (50, $[M - 258]^+$), 5333 (38, $[M - 344]^+$). Anal. calc. for $C_{267}H_{400}O_{129}$: C 56.52, H 7.11; found: C 56.37, H 7.01.

α-Benzyl-ω-hydroxytetrahexaconta{(R)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]} (**6b**). Following *General Procedure 2*, 0.70 g (0.012 mmol) of **6a** in 10 ml of CH_2Cl_2 were treated with 4 ml of CF_3COOH. The reaction was complete after 5 h at r.t. After the addition of 20 ml of 1,2-dichlorethane, the solvents were removed *in vacuo*. To remove trace amounts of CF_3COOH, the crude product was dissolved in CH_2Cl_2 and washed successively with sat. aq. $NaHCO_3$ (2×), 1N HCl, and brine. Removal of the solvent *in vacuo* and drying in high vacuum (40°/0.05 Torr) for 12 h yielded 0.68 g (99%) of a yellowish solid which was essentially pure (^{1}H-NMR). M.p. 153.5–154.5° $[\alpha]_{365} = +7.7$ ($c = 1.29$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1135*m*, 1100*m*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 7.35–7.20 (*m*, 5 arom. H); 5.30–5.20 (*m*, 63 H); 4.60–4.45 (*AB*, $J_{AB} = 11.6$, PhCH_2O); 4.05–3.95 (*m*, 1 H); $\nu_A = 2.61$, $\nu_B = 2.48$ (*AB* of *ABX*, $J_{AX} = 7.4$, $J_{BX} = 5.8$, $J_{AB} = 15.5$, 128 H); 1.45 (*s*, 9 H); 1.28 (*d*, $J = 6.3$, 192 H). ^{13}C-NMR (100 MHz): 169.13; 128.32; 127.62; 127.51; 71.93; 70.80; 67.98; 67.61; 67.41; 67.34; 42.12; 41.99; 40.80; 28.04; 19.85; 19.77.

α-Hydro-ω-hydroxytetrahexaconta{(R)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]} (**6d**). Following *General Procedure 2*, 1.0 g (0.18 mmol) of **6a** was deprotected using 30 ml of CH_2Cl_2 and 10 ml of CF_3COOH. After removal of the solvent and drying in high vacuum (50°/0.05 Torr) for 12 h, the yellowish solid was hydrogenated in 40 ml of CF_3CH_2OH with 0.1 g of Pd-C for 5 h at r.t. (*General Procedure 3*). Drying of the product for 18 h in high vacuum (50°/0.05 Torr) yielded 0.93 g (93%) of **6d**. The anal. data were determined from 0.5 g of this product purified by GPC on *Sephadex LH 60* in CH_2Cl_2. M.p. 161.0–161.5°. $[\alpha]_{365} = +7.1$ ($c = 0.99$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1130*m*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 5.40–5.20 (*m*, 63 H); 4.35–4.15 (*m*, 1H); $\nu_A = 2.60$, $\nu_B = 2.48$ (*AB* of *ABX*, $J_{AX} = 7.4$, $J_{BX} = 5.8$, $J_{AB} = 15.5$, 128 H); 1.28 (*d*, $J = 6.3$, 189 H); 1.22 (*d*, $J = 6.3$, 3 H). ^{13}C-NMR (100 MHz): 169.42; 169.15; 68.20; 67.72; 67.62; 43.25; 40.92; 40.81; 40.32; 19.99; 19.89; 19.77; 19.65. MALDI-MS: 5524 (79, $[M - 1]^+$), 5442 (100, $[M - 83]^+$), 5354 (82, $[M - 171]^+$), 5271 (64, $[M - 254]^+$), 5095 (42, $[M - 430]^+$). Anal. calc. for $C_{256}H_{386}O_{129}$: C 55.62, H 7.04; found: C 55.63, H 7.12.

α-Benzyl-ω-(tert-butoxy)hexanonaconta{(R)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]} (**7a**). Following *General Procedure 1*, the acid chloride of **6b** was prepared using 0.672 g (0.12 mmol) of **6b** and 1.2 g (9.2 mmol) of $(COCl)_2$ in 4 ml of CH_2Cl_2. Coupling of this acid chloride (in 4 ml of CH_2Cl_2) with 0.395 g (0.14 mmol) of **5c** and 0.2

g (2.5 mmol) of pyridine yielded 1.08 g of a white solid containing *ca.* 45% of **7a** (GPC). This solid was dissolved in 5 ml of CH_2Cl_2 and 0.3 ml of pyridine, and then 2.0 g (21 mmol) of 2,6-dichlorobenzoyl chloride in 10 ml of CH_2Cl_2 were added over 5 h and stirred for additional 2 h, as described in *General Procedure 4*. After the addition of half of the acid chloride, 66 mg (0.02 mmol) of **5c** were added again. Isolation of the product was performed by pouring the dark green mixture into 100 ml of Et_2O; the solid was separated by centrifugation to yield 1.12 g of a brownish solid containing *ca.* 60% **7a** (GPC). The anal. data were determined from 0.5 g of this product purified by GPC on *Sephadex LH 60* in CH_2Cl_2. M.p. 156–157°. $[\alpha]_{365} = +6.2$ ($c = 0.86$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1135*m*, 1100*m*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 7.35–7.20 (*m*, 5 arom. H); 5.30–5.20 (*m*, 95 H), 4.60–4.45 (*AB*, $J_{AB} = 11.6$, PhCH_2O); 4.05–3.95 (*m*, 1 H); $\nu_A = 2.61$, $\nu_B = 2.48$ (*AB* of *ABX*, $J_{AX} = 7.4$, $J_{BX} = 5.8$, $J_{AB} = 15.5$, 192 H); 1.45 (*s*, 9 H); 1.28 (*d*, $J = 6.3$, 288 H). ^{13}C-NMR (100 MHz): 169.15; 128.33; 127.63; 127.52; 71.94; 70.81; 67.99; 67.81; 67.62; 67.43; 67.35; 42.12; 42.00; 40.81; 28.05; 19.96; 19.77. MALDI-MS: 8439 (68, $[M + 14]^+$), 8365 (81, $[M - 89]^+$), 8275 (98, $[M - 179]^+$), 98), 8190 (100, $[M - 264]^+$), 8103 (92, $[M - 350]^+$), 8017 (87, $[M - 437]^+$), 7939 (74, $[M - 514]^+$), 7848 (64, $[M - 605]^+$). Anal. calc. for $C_{395}H_{592}O_{193}$: C 56.29, H 7.08; found: C 56.07, H 6.89.

α-Hydro-ω-hydroxyhexanonaconta{(R)-[oxy(1-methyl-3-oxopropane-1,3-diyl)]} (**7d**). Following *General Procedure 2*, 0.4 g (0.047 mmol) of crude **7a** were deprotected using 15 ml of CH_2Cl_2 and 5 ml of CF_3COOH. After removal of the solvent and drying in high vacuum (50°/0.05 Torr) for 12 h, the yellowish solid was hydrogenated in 25 ml of CF_3CH_2OH with 0.1 g of Pd-C over 5 h at r.t. (*General Procedure 3*). Drying of the product for 18 h in high vacuum (50°/0.05 Torr) yielded 0.37 g (92%) of **7d**. The anal. data were determined from 0.37 g of this product purified by GPC on *Sephadex LH 60* in CH_2Cl_2. M.p. 155.5–156.5°. $[\alpha]_D = +7.4$ ($c = 0.97$, CH_2Cl_2). IR (KBr): 2990*w*, 2970*w*, 1725*vs*, 1460*w*, 1380*m*, 1280*s*, 1230*m*, 1185*m*, 1130*m*, 1100*m*, 1060*m*, 980*m*. ^{1}H-NMR (400 MHz): 5.40–5.20 (*m*, 95 H); 4.35–4.15 (*m*, 1 H); $\nu_A = 2.60$, $\nu_B = 2.48$ (*AB* of *ABX*, $J_{AX} = 7.4$, $J_{BX} = 5.8$, $J_{AB} = 15.5$, 192 H); 1.28 (*d*, $J = 6.3$, 288 H). ^{13}C-NMR (100 MHz): 169.44; 169.16; 67.77; 67.63; 64.40; 43.26; 40.91; 40.81; 40.32; 22.54; 19.96; 19.77; 19.66. MALDI-MS: 8105 ($[M + 169]^+$). Anal. calc. for $C_{384}H_{578}O_{193}$: C 55.69, H 7.03; found: C 55.78, H 6.99.

REFERENCES

[1] M. Lemoigne, *Ann. Inst. Pasteur (Paris)* **1927,** *41,* 148, and earlier work cit. therein.

[2] E. A. Dawes, P. J. Senior, *Adv. Microbiol. Physiol.* **1973,** *10,* 203; E. A. Dawes, 'Microbial Energetics', Blakie, London, 1986.

[3] H.-M. Müller, D. Seebach, *Angew. Chem.* **1993,** *105,* 483; *ibid. Int. Ed.* **1993,** *32,* 477.

[4] *FEMS Microbiol. Rev.* **1992,** 103; 'Proceedings of the International Symposium on Bacterial Polyhydroxyalkanoates', Eds. H. G. Schlegel and S. Steinbüchel, Goltze-Druck, Göttingen, 1993.

[5] A. Steinbüchel, *Nachr. Chem. Tech. Lab.* **1991,** *39,* 10.

[6] P. A. Holmes, L. F. Wright, S. H. Collins, Eur. Pat. Appl. 1982, EP 52459 (*CA:* **1982,** *97,* 143146r); P. A. Holmes, F. Leonard, S. H. Collins, Eur. Pat Appl. 1983, EP 69497 (*CA:* **1983,** *98,* 141883a).

[7] R. N. Reusch, *FEMS Microbiol. Rev.* **1992,** *103,* 119.

[8] D. Seebach, A. K. Beck, U. Brändli, D. Müller, M. Przybylski, K. Schneider, *Chimia* **1990,** *44,* 112.

[9] Y. Shirakura, T. Fukui, T. Saito, Y. Okamoto, T. Narikawa, K. Koide, K. Tomita, T. Takemasa, S. Masamune, *Biochim. Biophys. Acta* **1986,** *880,* 46.

[10] D. A. Plattner, A. Brunner, M. Dobler, H.-M. Müller, W. Petter, P. Zbinden, D. Seebach, *Helv. Chim. Acta* **1993,** *76,* 2004.

[11] Y. Doi, 'Microbial Polyesters', VCH, Weinheim, 1990.

[12] H. M. Bürger, H.-M. Müller, D. Seebach, K. O. Börnsen, M. Schär, H. M. Widmer, *Macromolecules* **1993,** *26,* 4783.

[13] a) M. Rothe, in 'Chemistry and Physics of Macromolecules: Final Report of the Sonderforschungsbereich "Chemie und Physik der Makromoleküle"', Eds. E. W. Fischer, R. C. Schulz, and H. Sillescu, VCH, Weinheim, 1991, p. 30–60; b) K. S. Lee, G. Wegner, *Macromol. Chem., Rapid Commun.* **1985,** *6,* 203; I. Bidd, D. W. Holdup, M. C. Withing, *J. Chem. Soc., Perkin Trans. 1* **1987,** 2455; c) E. A. Adegoke, H. Ephraim-Bassey, D. J. Kelly, M. C. Whiting, *ibid.* **1987,** 2465; d) Z. Xu, J. S. Moore, *Angew. Chem.* **1993,** *105,* 261; *ibid. Int. Ed.* **1993,** *32,* 246.

[14] R. N. Reusch, H. Sadoff, *Proc. Natl. Acad. Sci. U.S.A.* **1988,** *85,* 4176.

[15] A. Ballistreri, D. Garozzo, M. Giuffrida, G. Impallomeni, G. Montaudo, *Macromolecules* **1989,** *22,* 2107; S. Coulombe, P. Schauwecker, R. H. Marchessault, B. Hauttecœur, *ibid.* **1978,** *11,* 279.

[16] F. E. Küng, US-Pat. 1944, 2361036 (*CA*: **1944,** *38,* 6301); H. Morikawa, R. H. Marchessault, *Can. J. Chem.* **1981,** *59,* 2306; N. Grassi, E. J. Murray, P. A. Holmes, *Polym. Degrad. Stabil.* **1984,** *6,* 47, 95, 127; M. Kunioka, Y. Doi, *Macromolecules* **1990,** *23,* 1933; R. H. Marchessault, S. Coulombe, H. Morikawa, K. Okamura, J. F. Revol, *Can. J. Chem.* **1981,** *59,* 38.
[17] A. Keller, 'Reports on Progress in Physics', Ed. C. I. Pedersen, The Institute of Physics and the Physical Society, London, 1968, Vol. 31, p. 632.
[18] Faraday Discussions of the Royal Society of Chemistry 1970, 68, passim.
[19] D. J. Blundell, A. Keller, T. M. Connor, *J. Polym. Sci. A-2* **1967,** *5,* 991.
[20] T. Williams, D. J. Blundell, A. Keller, I. M. Ward, *J. Polym. Sci. A-2* **1968,** *6,* 1613.
[21] A. Keller, E. Martuscelli, D. J. Priest, Y. Udagawa, *J. Polym. Sci. A-2* **1971,** *9,* 1807.
[22] E. J. Welland, J. Stejny, A. Halter, A. Keller, *Polym. Commun.* **1989,** *30,* 302.
[23] S. J. Organ, A. Keller, *J. Polym. Sci. Part B: Polym. Phys.* **1987,** *25,* 2409.
[24] D. Seebach, A. Brunner, H. M. Bürger, R. N. Reusch, J. Schneider, *Eur. J. Biochem.*, in press.
[25] R. S. Cahn, C. K. Ingold, V. Prelog, *Angew. Chem.* **1966,** *78,* 413; *ibid. Int. Ed.* **1966,** *5,* 385; V. Prelog, G. Helmchen, *Angew. Chem.* **1982,** *94,* 614; *ibid. Int. Ed.* **1982,** *21,* 567.
[26] R. N. Reusch, *Proc. Soc. Exp. Biol. Med.* **1989,** *191,* 377.
[27] a) M. Yokuchi, Y. Chatani, H. Tadokoro, K. Teranishi, H. Tani, *Polymer* **1973,** *14,* 267; b) J. Cornibert, R. H. Marchessault, *J. Mol. Biol.* **1972,** *71,* 735; J. Cornibert, R. H. Marchessault, *Macromolecules* **1975,** *8,* 296; c) S. Brückner, S. V. Meille, L. Malpezzi, A. Cesaro, L. Navarini, R. Tombolini, *ibid.* **1988,** *21,* 967.
[28] W. Klyne, V. Prelog, *Experientia* **1960,** *16,* 521.
[29] J. Dale, 'Stereochemie und Konformationsanalyse', VCH, Weinheim, 1978.
[30] D. M. Engelman, T. A. Steitz, A. Goldman, *Ann. Rev. Biophys. Biophys. Chem.* **1986,** *15,* 321.
[31] J. Deisenhofer, H. Michel, *Angew. Chem.* **1989,** *101,* 872; *ibid. Int. Ed.* **1989,** *28,* 829, *cf.* Chapt. 5.2.
[32] A. L. Lehninger, 'Biochemie', Verlag Chemie, Weinheim, 1975; D. E. Metzler, 'Biochemistry', Academic Press, New York, 1977.
[33] M. Dobler, MacMoMo-Molecular Modelling Program Version II 1.0, Laboratory of Organic Chemistry, ETH-Zürich, 1993.
[34] H. Einspahr, C. E. Bugg, in 'Calcium-Binding Proteins and Calcium Function', Elsevier North-Holland Inc., New York, 1977, p. 13.
[35] D. Seebach, H. M. Bürger, D. A. Plattner, R. Nesper, T. Fässler, *Helv. Chim. Acta* **1993,** *76,* 2581.
[36] L. Bramble, R. N. Reusch, *Science,* in press.
[37] C. Miller, *Physiol. Rev.* **1983,** *63,* 1209.