Synthesis and NMR Analysis in Solution of Oligo(3-hydroxyalkanoic acid) Derivatives with the Side Chains of Alanine, Valine, and Leucine (β-Depsides): Coming Full Circle from PHB to β-Peptides to PHB

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Oligomers of 3-hydroxyalkanoic acids that contain two, three, and six residues with and without O-terminal (*t*Bu)Ph_2Si and C-terminal $PhCH_2$ protection have been synthesized in such a way that the side chains on the oligoester backbone were those of the proteinogenic amino acids Ala (Me), Val ($CHMe_2$), and Leu (CH_2CHMe_2). The enantiomerically pure 3-hydroxyalkanoates were obtained by *Noyori* hydrogenation of the corresponding 3-oxo-alkanoates with [Ru((*R*)-binap)Cl_2](binap = 2,2′bis(diphenylphosphanyl)-1,1′-binaphthalene)/H_2 (*Scheme 1*), and the coupling was achieved under the conditions (pyridine/$(COCl)_2$, CH_2Cl_2, −78°) previously employed for the synthesis of various oligo(3-hydroxybutanoic acids) (*Schemes 2* and *3*). The *Cotton* effects in the CD spectra of the new oligoesters provided no hints about chiral conformation (*cf.* a helix) in MeOH, MeCN, octan-1-ol, or CF_3CH_2OH solutions (*Figs. 1* and *2*). Detailed NMR investigations in $CDCl_3$ solution (*Figs. 3–6*, and *Tables 1–5*) of the hexa(3-hydroxyalkanoic acid) with the side chains of Val (HC), Ala (HB), Leu (HH), Val, Ala, Leu (from O- to C-terminus; **3**) gave, on the NMR time-scale, no evidence for the presence of any significant amount of a 2_1- or a 3_1-helical conformation, comparable to those identified in stretched fibers of poly[(*R*)-3-hydroxybutanoic acid], or in lamellar crystallites and in single crystals of linear and cyclic oligo[(*R*)-3-hydroxybutanoic acids], or in the corresponding β-peptide(s) (the oligo(3-aminoalkanoic acid) analogs; **1**–**3**). Thus, the extremely high flexibility (averaged or 'random-coil' conformation) of the polyester chain (CO–O rotational barrier *ca.* 13 kcal/mol; no hydrogen bonding), as compared to polyamide chains (CO–NH barrier *ca.* 18 kcal/mol; hydrogen bonding) has been demonstrated once again. The possible importance of this structural flexibility, which goes along with amphiphilic properties, for the role of PHB in biology, in evolution, and in prebiotic chemistry is discussed. Structural similarities of natural potassium-channeling proteins and complexes of oligo(3-hydroxybutanoates) with Na^+, K^+, or Ba^{2+} are alluded to (*Figs. 7–9*).

1. Indroduction. – The short-chain version of poly[(*R*)-3-hydroxybutanoic acid] (c-PHB), **1**, has been detected in numerous biological systems, and we believe that it is fair to say that the biopolymer PHB is present in all living organisms[3]). The low-molecular-weight PHB has been shown to cause phospholipid membranes to become permeable for cations, such as Na, K, Rb, Ca or Ba, under voltage-driven (patch-clamp experiments [5]) and under concentration-driven (artificial vesicles [6]) conditions. Furthermore, a Ca polyphosphate-PHB complex consisting of *ca.* 140 HB and 70 phosphate units and containing *ca.* 35 Ca^{2+} ions has been identified as a Ca-specific ion

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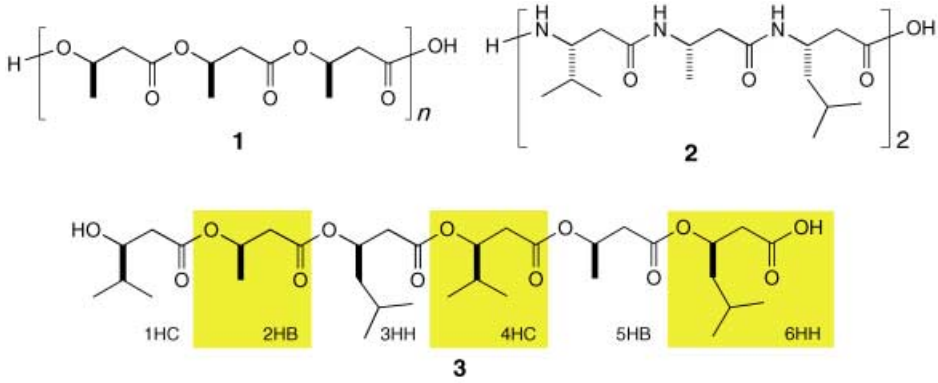

For the description of the NMR spectra with full assignement of all residues in the chain, we needed to have abbreviations to designate the six residues of **3** unambiguously; we chose 1- and 4-HC, 2- and 5-HB, and 3- and 6-HH (**h**ydroxy-**c**aproic, -**b**utyric and -**h**eptanoic acid).

channel, extractable from the inner cell wall of genetically competent *E. coli* [7][8]. c-PHB and polyphosphate have recently been identified as part of the microbial KcsA potassium channel, in which an amino acid residue bears the polyester chain (complexing $Ca \cdot PP_i$) [9].

In view of the biological importance of PHB, it is essential to learn as much as possible about its structure. Two distinct folding patterns of the polyester chain have been characterized: a 2_1 helix in stretched fibers [10] and in lamellar crystallites [11][12] of the polymer, and both 2_1 and 3_1 helices in crystals of cyclic oligomers ('oligolides') of HB [13–16] (*Fig. 1,a,* and *b*). In contrast, all attempts to find a preferred conformation (secondary structure) of the polyester chain in solution have failed so far; among the methods used were CD [17][18] and NMR [17][4]) spectroscopy, as well as FRET[5]) measurements [21]. While there are indications for the presence of secondary structures in the ensemble of molecules on the very short timescale of UV/VIS spectroscopy [17][18][21], no preferred conformation could be detected on the slow NMR timescale [17]. On the other hand, inspection of the 3_1 helix (*Fig. 1,b*) indicated that replacement of the chain-bound O-atoms by NH groups would lead to NH···O=C H-bonding, and thus to stabilization of the helix. This was, indeed, the case (*Fig. 1,c*), and has led to our entry into the field of β-peptides [22], systematic investigations of which showed that the 3_1 or 3_{14} helix observed in solutions of the β-hexapeptide H-(β-HVal-β-HAla-β-HLeu)$_2$-OH (**2**) may be due to preferred backbone conformations and not only due to multiple H-bonding [22]. Thus, we thought it worthwhile to have a look at a *β-hexadepside*, the O-analog **3** of the β-hexapeptide[6]),

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4) ^{13}C-NMR Experiments with solid-phase copolymers of poly(β-hydroxybutyrate-co-β-hydroxyvalerate) (P(3 HB-3 HV)) [19] or P(3 HB-3 HV) in chloroform solution [20] have also been reported.

5) FRET = Fluorescence Resonance Energy Transfer.

6) A β-depsipeptide consisting of six β-amino acids and a central β-hydroxybutanoic acid (HB) residue has been previously described [23]. Also, chimeric, MHC-binding oligomers, which, contain α- and β-amino, as well as β-hydroxy acids, have been synthesized [24]. Oligomers of β-hydroxy acids with proteinaceous side chains have not previously been reported; they are β-depsides, homologs of α-depsides, which have been prepared from lactic acid (the Ala analog), mandelic acid (the phenylglycine analog), and 2-hydroxy-3-methylbutanoic acid (the Val analog); no α-depsidic structure has been published; for CD spectra, see [25].

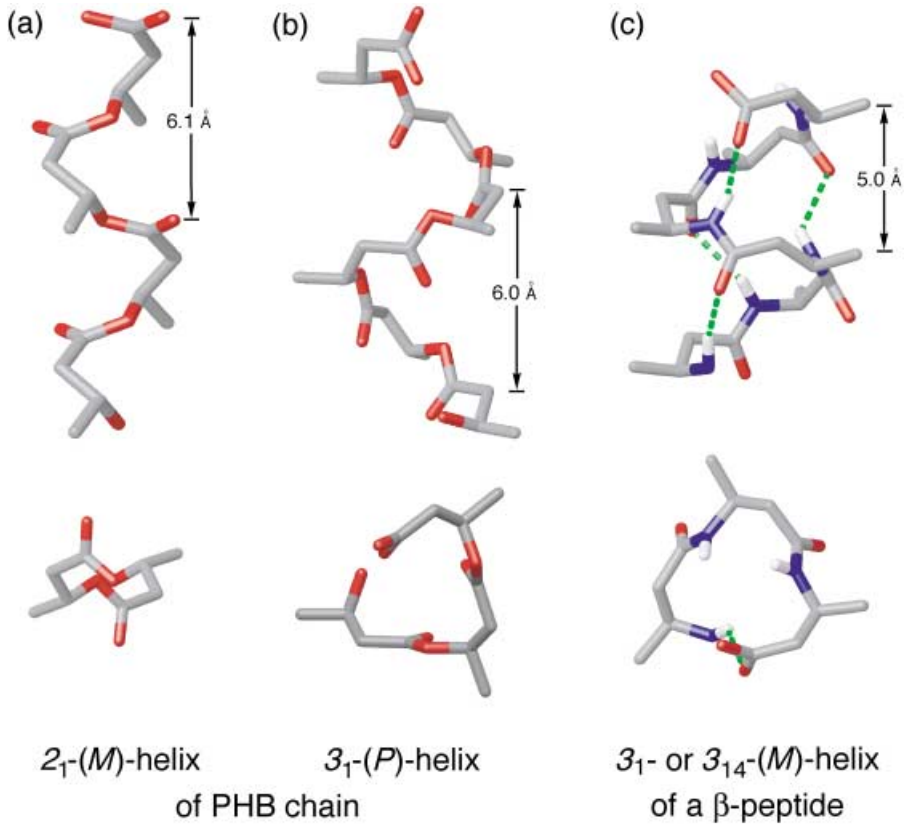

Fig. 1. *Models of the different helical conformations of PHB and β^3-oligopeptides. a*) The 2_1 helix, *b*) the 3_1 helix of a PHB chain, *c*) the 3_1 or 3_{14} helix of a β^3-peptide.

with different and larger side chains, which hopefully better stabilize certain backbone conformations[7]) and allow easier NMR assignments of the sequential residues due to increased resolution as compared to HB oligomers with identical Me groups[8]).

2. Preparation of the Hexakis(3-hydroxy-alkanoic acid) 3. – Linear and cyclic oligomers of (*R*)-3-hydroxyvalerate (HV) and (*R*)-3-hydroxybutyrate (HB) have been previously synthesized by *Seebach* and co-workers [12–16][28]. While the enantiomerically pure building blocks required for these oligomers were obtained by hydrolysis of the biopolymer PHB/PHV [29], β-hydroxy acid derivatives with substituents other than Me or Et in the β-position had to be prepared by enantioselective synthesis. The methodology (*Scheme 1*) chosen for that purpose, *i.e.*,

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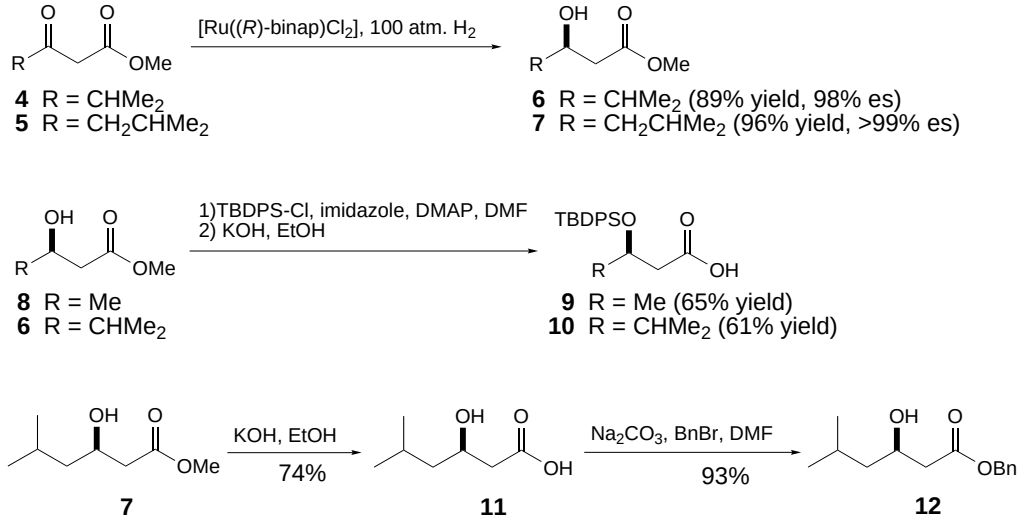
7) For the preferred confomations of various types of esters, see [26].

8) Oligomer **3** is an oligo(3-hydroxyalkanoic acid) (OHB) with different side chains in specific positions. This kind of OHB has not been previously described, while there is a host of literature on poly(3-hydroxyalkanoic acids) (PHA) with uniform or irregularly placed side chains [27].

hydrogenation of β-keto esters[9]) with the *Noyori* catalyst [Ru((*R*)-binap)Cl_2] [31] has previously been used by us for the preparation of (*S*)-3-hydroxybutyrate [16]. The Ru-binap catalyst was prepared *in situ*, according to a procedure by *Genêt et al.* [32], and the hydrogenation of β-keto esters **4** and **5** was carried out under 100 atm H_2 pressure at ambient temperature to yield the products **6** and **7**, respectively, with enantiomer purities above 98%[10]) in essentially quantitative yield. For the preparation of the building blocks **9** and **10**, the hydroxy esters **8** and **6**, respectively, were first *O*-silylated and then saponified. Conversion of the methyl ester **7** to the benzyl ester **12** occurred *via* the free hydroxycarboxylic acid **11**, which was subsequently benzylated (*Scheme 1*).

Scheme 1



TBDPS = (*t*-Bu)Ph_2Si

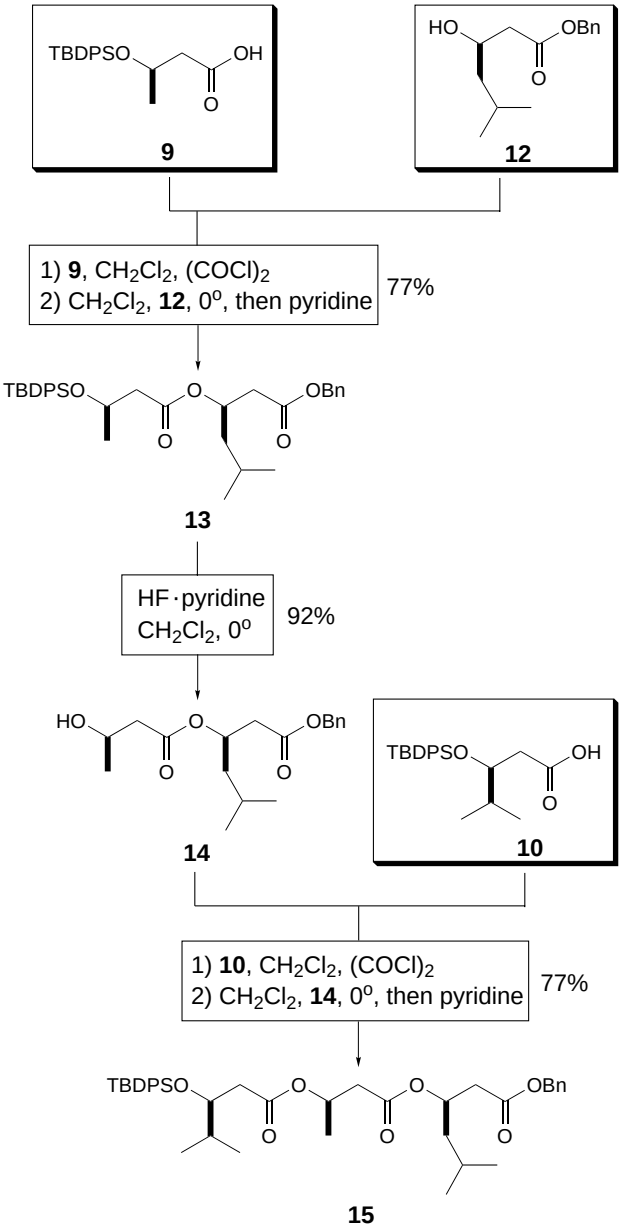
A sequence of stepwise coupling and fragment coupling (*cf.* HB oligomers with [17] and without isotopic labeling [13–16][28]) led from the monomeric building blocks **9**, **10**, and **12** through the dimeric (*i.e.*, **13**, **14**) and trimeric (*i.e.*, **15**–**17**) intermediate products to the protected and unprotected target compounds, **18** and **3** (*Schemes 2* and *3*).

3. CD Spectra of the Oligomers 3, 13, 15, and 18. – Like the oligomers of (*R*)-3-hydroxybutyric acid (OHBs), consisting of fewer than ten residues, the oligo(3-hydroxyakanoic acid) derivatives described here show a weak positive *Cotton* effect, which is slightly red-shifted (213–218 *vs.* 212 nm, *Fig. 2,a*). The unprotected 'hexamer' **3** has a molar ellipticity Θ of 4500 in octan-1-ol and in MeCN solution; this value

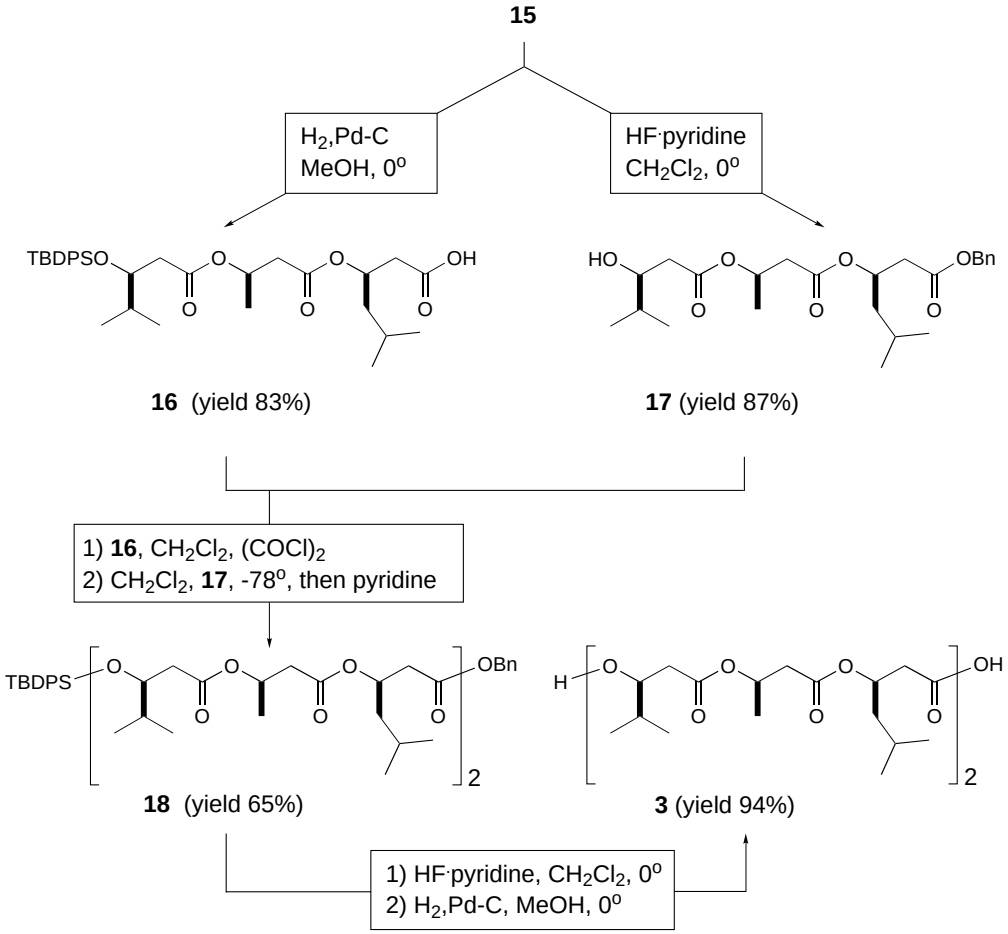
[9]) The β-keto esters were obtained by acylation of *Meldrum*'s acid, followed by alcoholysis, according to the procedure of *Yonemitsu* and co-workers [30].

[10]) Determined by NMR, after derivatization with *Mosher*'s ester [33].

Scheme 2


TBDPSO
OH
O
9
HO
OBn
O
12
1) 9, CH2Cl2, (COCl)2
2) CH2Cl2, 12, 0°, then pyridine
77%
TBDPSO
O
OBn
13
HF·pyridine
CH2Cl2, 0°
92%
HO
14
TBDPSO
OH
10
1) 10, CH2Cl2, (COCl)2
2) CH2Cl2, 14, 0°, then pyridine
77%
15

Scheme 3



increases as the polarity of the solvent increases: $\Theta = 7000$ and 8500 in MeOH and CF_3CH_2OH, respectively (*Fig. 2,b*).

The higher intensities of the *Cotton* effects from the fully or partially protected derivatives (*Fig. 2,a*) is due to the presence of Ph groups in the O- and C-terminal protection ((*t*-Bu)Ph_2Si, CH_2Ph). The CD spectrum of the β-peptide analog **2** of the oligoester **3** gives rise to a much stronger *Cotton* effect ($\Theta = 40000$ at 216 nm in MeOH [23–25][34]). Thus, the CD spectra provide no indication for the presence of a helical secondary structure in the solution of the oligo(hydroxy acid) **3**.

4. NMR Investigation of the Hexakis(hydroxy acid) 3. – *Spectral Assignment*. The ^{1}H-NMR chemical shifts have been assigned from a P.E.COSY spectrum (*Fig. 3*) together with a ROESY spectrum (data not shown), annotations are given in *Fig. 3* (*top:* annotated units 1 to 3; *bottom:* annotated units 4 to 6). Intra-residual resonance

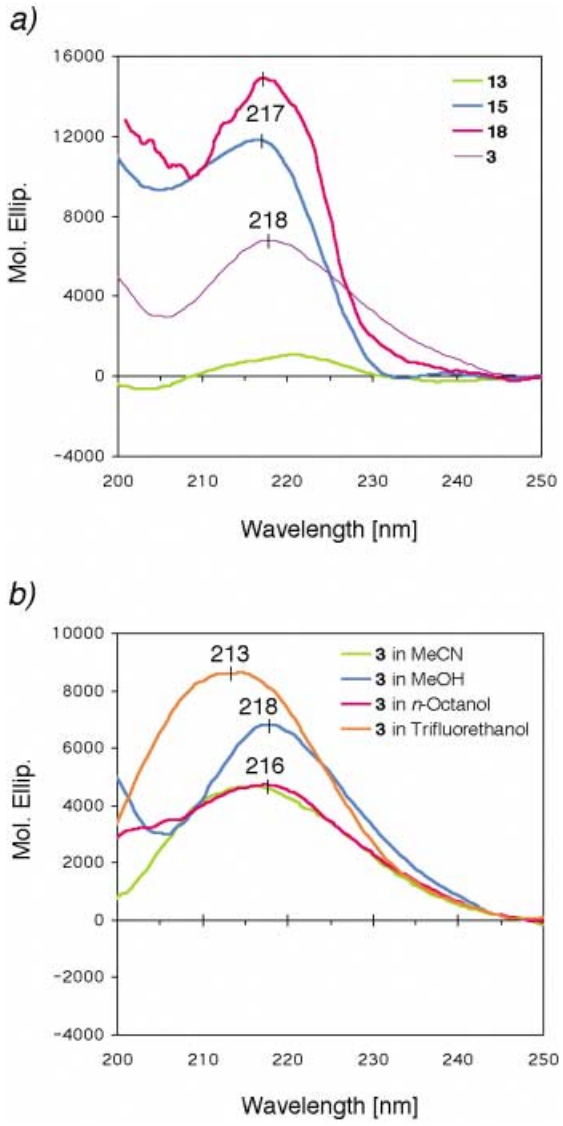


Fig. 2. *Non-normalized CD spectra of the oligoesters* **3**, **13**, **15**, *and* **18**. *a*) In MeOH, *b*) CD Spectra of oligoester **3** in different solvents.

assignment has been performed on the basis of direct connectivities and residue-type specific resonance patterns in the P.E.COSY spectrum. Sequential assignment has been derived from the ROESY spectrum. The starting point of the assignment was the OH terminal 1 HC, the C(3)H proton of which is shifted upfield with respect to the other residues. Direct $i,i+1$ ROE connectivities could be observed for all residues, which enabled us to completely derive the sequential connectivity. ^{13}C-NMR Chemical-shift assignment has been derived from the ^{1}H,^{13}C-HSQC (*Fig. 4*) for all resonances except

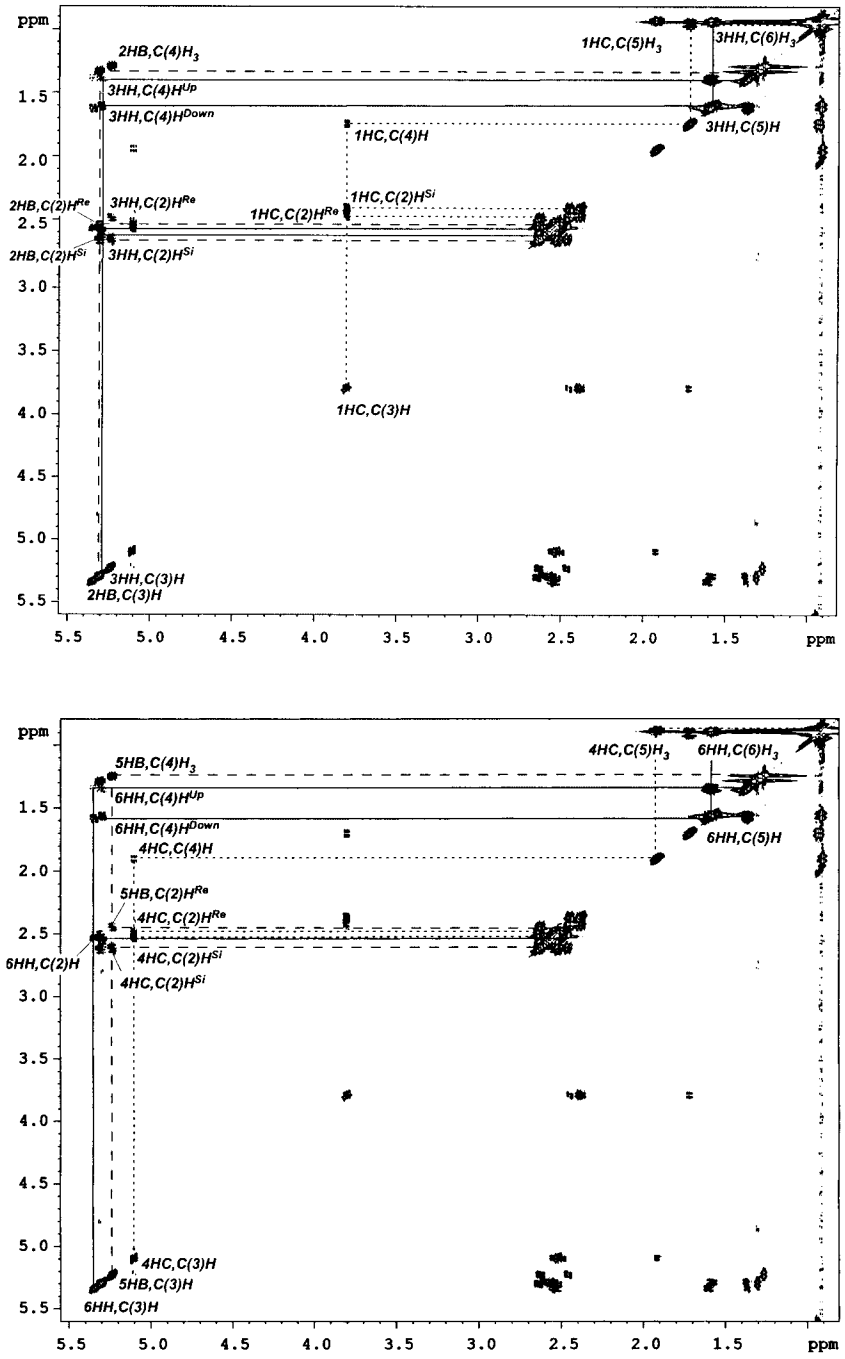


Fig. 3. *P.E.COSY Spectrum of* **3**, *assignment of the first three* (top) *and the last three units* (bottom). The HH $C(4)H_2$ protons have not been stereospecifically assigned and are, therefore, denoted according to their relative chemical shift (*Up* = upfield, *Down* = downfield).

the C=O moieties, which have been assigned from an $^{1}H,^{13}C$-HMBC spectrum. The chemical-shift assignments are listed in *Table 1*. The resonances of all repetition units are resolved except for 3 HH and 6 HH, and 2 HB and 5 HB, which overlap partially with one another. In addition, the 3 HH unit shows interresidual signal overlap with 2 HB, which renders the analysis of this residue more difficult.

Conformational Analysis: Assignment of the Diastereotopic C(2)H$_2$ Protons. To stereospecifically assign the diastereotopic C(2)H$_2$ protons, vicinal coupling constants have been measured and interpreted for the hexamer **3**. In addition, H,H-distance information has been derived from integration of intra-residual ROE (rotating-frame *Overhauser* enhancement) cross-peaks. ^{3}J(C(2)H$_2$,C(3)H) coupling constants extracted from the P.E.COSY spectrum (*Fig. 3*) and qualitative ^{3}J(C(2)H$_2$,C(4)) coupling constants derived from the $^{1}H,^{13}C$-HMBC spectrum (*Fig. 5*) are given in *Table 2* together with the ratios $R_{\mathrm{ROE}}^{Up/Down} = \dfrac{\mathrm{ROE(C(2)H^{Down}, C(}i\mathrm{)H)}}{\mathrm{ROE(C(2)H^{Up}, C(}i\mathrm{)H)}}$ for $i = 3,4$ of the intra-residual C(2)H$_2$,C(3)H and the C(2)H$_2$,C(4)H ROEs. Here, the annotations *Down* and *Up* signify the relative chemical shift of the two C(2)H$_2$ protons. The three possible rotamers (*ap,* (−)-*sc*, and (+)-*sc*) are shown in *Fig. 6*, together with the characteristic relative sizes of ^{3}J coupling constants and ROE intensities.

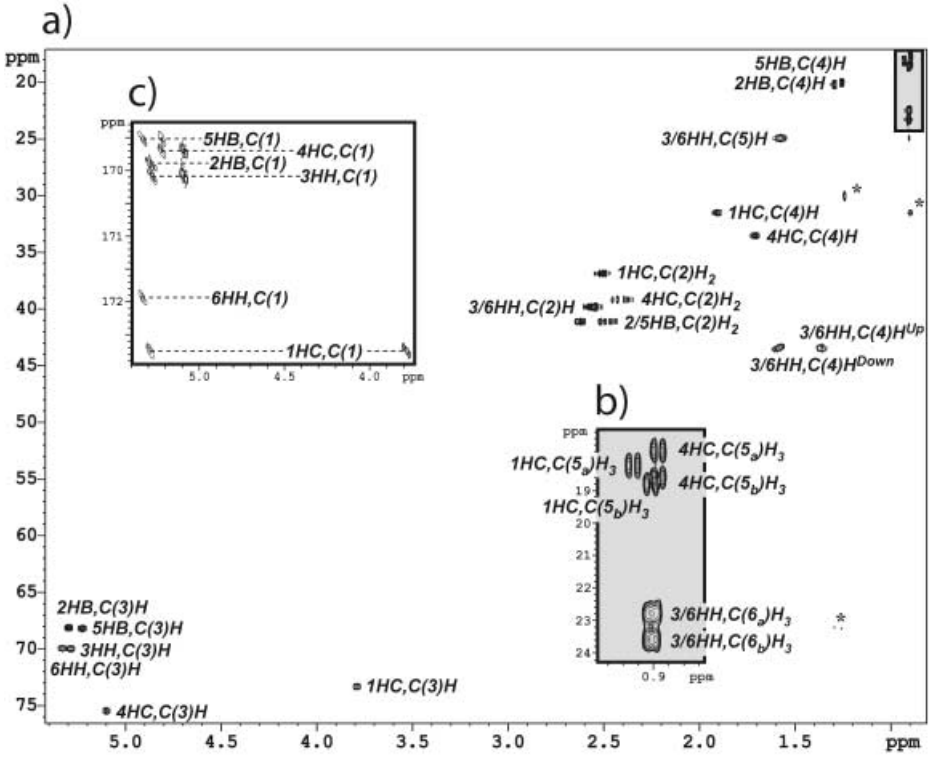


Fig. 4. a) *Natural-abundance $^{1}H,^{13}C$-HSQC spectrum of* **3**. The grey-shaded region has been magnified in *b*. Asterisks indicate impurities *c*) C(3)H/(C1) region of a natural-abundance $^{1}H,^{13}C$-HMBC spectrum. C(1) assignments are given.

Table 1. *^1H and ^{13}C Chemical-Shift Assignment for* **3**

Residue	H-Atom	Chemical shift [ppm]	C-Atom	Chemical shift [ppm]
1 HC			C(1)	172.75
	$C(2)H^{Re}$	2.43	C(2)	36.48
	$C(2)H^{Si}$	2.36		
	C(3)H	3.78	C(3)	72.94
	C(4)H	1.70	C(4)	31.10
	$C(5_1)H_3$	0.89	$C(5_1)$	18.29
	$C(5_2)H_3$	0.91	$C(5_2)$	17.72
2 HB			C(1)	169.92
	$C(2)H^{Re}$	2.50	C(2)	40.73
	$C(2)H^{Si}$	2.62		
	C(3)H	5.29	C(3)	67.73
	$C(4)H_3$	1.28	C(4)	19.87
3 HH			C(1)	170.12
	$C(2)H^{Re}$	2.58	C(2)	39.28
	$C(2)H^{Si}$	2.52		
	C(3)H	5.27	C(3)	69.59
	$C(4)H^{Up}$	1.34	C(4)	42.94
	$C(4)H^{Down}$	1.56		
	C(5)H	1.56	C(5)	24.59
	$C(6_1)H_3$	0.89	$C(6_1)$	22.88
	$C(6_2)H_3$	0.89	$C(6_2)$	22.15
4 HC			C(1)	169.72
	$C(2)H^{Re}$	2.52	C(2)	38.83
	$C(2)H^{Si}$	2.47		
	C(3)H	5.08	C(3)	75.08
	C(4)H	1.90	C(4)	33.15
	$C(5_1)H_3$	0.89	$C(5_1)$	18.12
	$C(5_1)H_3$	0.89	$C(5_2)$	17.22
5 HB			C(1)	169.54
	$C(2)H^{Re}$	2.44	C(2)	40.73
	$C(2)H^{Si}$	2.61		
	C(3)H	5.22	C(3)	67.78
	$C(4)H_3$	1.25	C(4)	19.70
6 HH			C(1)	171.74
	$C(2)H^{Re}$	2.52	C(2)	39.38
	$C(2)H^{Si}$			
	C(3)H	5.33	C(3)	69.49
	$C(4)H^{Up}$	1.43	C(4)	43.24
	$C(4)H^{Down}$	1.56		
	C(5)H	1.56	C(5)	24.59
	$C(6_1)H_3$	0.89	$C(6_1)$	22.88
	$C(6_2)H_3$	0.89	$C(6_2)$	22.15

The stereospecific assignments of the diastereotopic $C(2)H_2$ protons has been performed as described below for each unit but for 6 HH, for which the chemical shifts of the two $C(2)H_2$ protons are degenerate:

1 HC. $^3J(C(2)H^{Up},C(3)H)$ is significantly larger than $^3J(C(2)H^{Down},C(3)H)$ (9.7 compared to 2.7 Hz), indicating that $C(2)H^{Up}$ is in an antiperiplanar orientation with

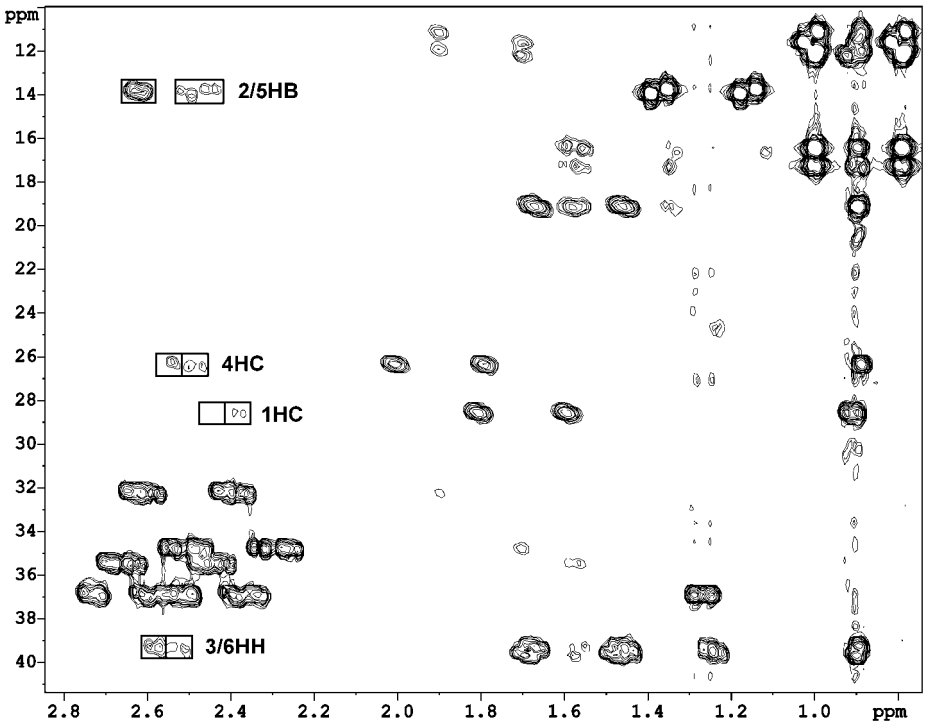
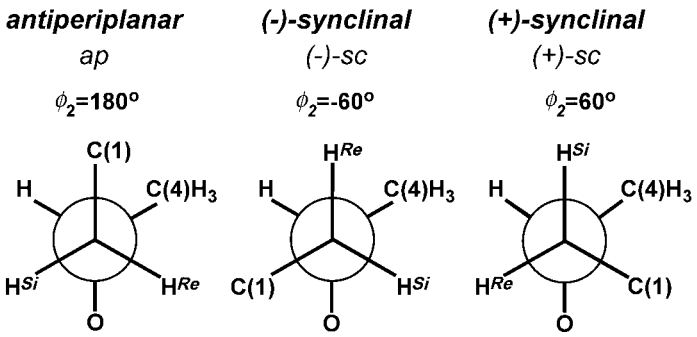


Fig. 5. *Natural-abundance $^{1}H,^{13}C$-HMBC spectrum of* **3**. C(2)H_2,C(4) Cross-peaks are indicated.

Table 2. *$^{3}J(C(2)H_2,C(3)H)$ Coupling Constants Extracted from the P.E.COSY Spectrum of* **3**, *Relative $^{3}J(C(2)H_2,CB)$ Coupling Constants from the HMBC Spectrum.* +: Larger value, −: smaller value, n.d.: not determined. $R_{\mathrm{ROE}}^{Up/Down} = \dfrac{\mathrm{ROE(C(2)H^{Down},\ C(}i\mathrm{)H)}}{\mathrm{ROE(C(2)H^{Up},\ C(}i\mathrm{)H)}}$ for $i = 3$ and $i = 4$ are given.

Residue	Atom identity	Stereo-specific assign-ment	^{3}J(C(4),C(2)H_2) [Hz]	^{3}J(C(4),C(2)H_2) [Hz]	$\frac{\mathrm{ROE(C(2)H^{Down},\ C(4)H)}}{\mathrm{ROE(C(2)H^{Up},\ C(4)H)}}$	$\frac{\mathrm{ROE(C(2)H^{Down},\ C(3)H)}}{\mathrm{ROE(C(2)H^{Up},\ C(3)H)}}$
1 HC	C(2)H^{Down}	C(2)H^{Re}	2.7	−	0.97	1.89
	C(2)H^{Up}	C(2)H^{Si}	9.7	+		
2 HB	C(2)H^{Down}	C(2)H^{Si}	7.6	+	0.81	0.28
	C(2)H^{Up}	C(2)H^{Re}	4.6	−		
3 HH	C(2)H^{Down}	C(2)H^{Re}	5.3	+	n.d.	0.46
	C(2)H^{Up}	C(2)H^{Si}	< 5.3	−		
4 HC	C(2)H^{Down}	C(2)H^{Re}	5.9	+	n.d.	n.d.
	C(2)H^{Up}	C(2)H^{Si}	4.9	−		
5 HB	C(2)H^{Down}	C(2)H^{Si}	7.4	+	0.74	0.71
	C(2)H^{Up}	C(2)H^{Re}	4.9	−		
6 HH	C(2)H^{Down}	C(2)H^{Si}	n.d.	n.d.	n.d.	n.d.
	C(2)H^{Up}	C(2)H^{Re}	n.d.	n.d.		



	antiperiplanar ap ϕ_2=180°	(-)-synclinal (-)-sc ϕ_2=-60°	(+)-synclinal (+)-sc ϕ_2=60°
^3J(C(3)H,C(2)H^{Si})	+	**+**	+
^3J(C(3)H,C(2)H^{Re})	**+**	+	+
^3J(C(4),C(2)H^{Si})	**+**	+	+
^3J(C(4),C(2)H^{Re})	+	+	**+**
ROE(C(3)H,C(2)H^{Si})	**+**	+	**+**
ROE(C(3)H,C(2)H^{Re})	+	**+**	**+**
ROE(C(4)H,C(2)H^{Si})	+	**+**	**+**
ROE(C(4)H,C(2)H^{Re})	**+**	**+**	+

Fig. 6. *The three staggered conformers about the angle* ϕ_2. Relative ^3J coupling constants and ROE intensities are given. Bold plus: Large relative value; thin plus: small relative value.

respect to C(3)H in the predominant conformation. Since both heteronuclear ^3J(C(2)H$_2$,C(4)) coupling constants are small (as evidenced by the very weak peaks in the HMBC spectrum; *Fig. 6*), the C(2)H$_2$ protons are both oriented *synclinal* to C(4). Hence, the predominant conformation is (−)-*sc*. The ratio $R_{\mathrm{ROE}}^{Up/Down}$ of the two C(2)H$_2$,C(4)H ROEs is close to 1, and $R_{\mathrm{ROE}}^{Up/Down}$ for the two C(2)H$_2$,C(3)H ROEs is *ca.* 2. These results are in agreement with the relative H,H distances predicted for the (−)-*sc* conformation. Based on this analysis, C(2)H^{Up} is C(2)H^{Si}, C(2)H^{Down} is C(2)H^{Re}.

2 HB/5 HB. For both residues, ^3J(C(2)H^{Down},C(3)H) is significantly larger than ^3J(C(2)H^{Up},C(3)H) (7.6 and 7.4 Hz compared to 4.6 and 4.9 Hz for 2 HB and 5 HB, respectivly), hence C(2)H^{Down} is in an antiperiplanar orientation with respect to C(3)H

in the predominant conformation for both units. Both $^3J(C(2)H_2,C(4))$ coupling constants are small. This indicates a predominant (−)-*sc*-conformation with a reversed diastereotopic assignment of the $C(2)H_2$ protons as compared to 1 HC: $C(2)H^{Up}$ is $C(2)H^{Re}$, $C(2)H^{Down}$ is $C(2)H^{Si}$.

The observed differences in $R_{ROE}^{Up/Down}$ for the two HB units, however, are not in agreement with only a pure (−)-*sc*-conformation. This observation has not been further analyzed due to the insensitivity of ROEs to conformational averaging.

3 HH/4 HC. The two $^3J(C(2)H_2,C(3)H)$ coupling constants are almost equal for 4 HC (5.9 and 4.9 Hz), indicating that both $C(2)H_2$ protons are in a *synclinal* orientation with respect to C(3)H. The overlap of the 3 HH $C(2)H^{Up}$,C(3)H cross-peak makes a quantification of the $^3J(C(3)H,C(2)H^{Up})$ coupling constant impossible. Qualitative analysis, however, shows that $^3J(C(2)H^{Up},C(3)H)$ is smaller than $^3J(C(2)H^{Down},C(3)H)$ in this unit. The 3 HH $^3J(C(3)H,C(2)H_2)$ coupling constants are of similar size as the coupling constants found for 4 HC. Both $^3J(C(2)H_2,C(4))$ coupling constants are small for both units. The $C(2)H_2$ proton with the slightly larger $^3J(C(3)H,C(2)H_2)$ has also the larger $^3J(C(4),C(2)H_2)$ coupling constant, which shows that the predominant conformation is (+)-*sc*, and *ap* is second most populated in both units. Therefore, $C(2)H^{Down}$ is $C(2)H^{Re}$ and $C(2)H^{Up}$ is $C(2)H^{Si}$.

NMR Analysis: Backbone Angle ϕ_2. The populations of the three conformations about the angle ϕ_2 based on the stereospecific assignment, as well as a detailed description of the coupling-constant analysis are given in *Table 3*. $^3J(C(2)H_2,C(3)H)$ Coupling constants and the conformation about the angle ϕ_2 are averaged for all units compared to $^3J(C(2)H_2,C(3)H)$ coupling constants derived from the generalized *Karplus* equation [35] (see caption of *Table 3*).

Table 3. *$^3J(C(2)H_2,C(3)H)$ Coupling Constants and Resulting Populations of the Three Staggered Conformations about the Angle* ϕ_2. The populations P have been calculated using the *Pachler* analysis equations: $^3J(C(2)H^{Re},C(3)H) = P_{(+)\text{-}sc}J_{(+)\text{-}sc} + P_{ap}J_{ap} + P_{(-)\text{-}sc}J_{(-)\text{-}sc}$, $^3J(C(2)H^{Si},C(3)H) = P_{ap}J_{(+)\text{-}sc} + P_{(-)\text{-}sc}J_{ap} + P_{(+)\text{-}sc}J_{(-)\text{-}sc}$ and $1 = P_{ap} + P_{(-)\text{-}sc} + P_{(+)\text{-}sc}$. J_{ap}, $J_{(-)\text{-}sc}$ and $J_{(+)\text{-}sc}$ can be obtained from the generalized *Karplus* equation [35]: $^3J(H,H) = 13.7\cos^2\phi + 0.73\cos\phi + \Sigma_i\Delta c_i[0.56 - 2.47\cos^2(z_i\phi + 16.9\ |\Delta c_i|)]$. In this equation, ϕ is the dihedral angle between the two protons, z_i is the orientation factor of substituent i, Δc_i is the electronegativity difference between substituent i and the respective proton. Not only the direct substituents ia are considered but also the atoms bound to the i,a substituents (i,b). Thus, Δc_i is determined according to $\Delta c_i = \Delta c_{i,a} - 0.14\ S\ \Delta c_{i,b}$ (O: Δc_i = 1.3, C: Δc_i = 0.4, H: Δc_i = 0.0). The $^3J(C(2)H_2,C(3)H)$ coupling constant are 11.63 for ap, 3.55 for (+)-*sc*, and 1.97 Hz for (−)-*sc*.

Residue	P_{ap} [%]	$P_{(-)\text{-}sc}$ [%]	$P_{(+)\text{-}sc}$ [%]
1 HC	4.5	77.0	18.5
2 HB	23.7	54.8	21.5
3 HH	–	–	–
4 HC	33.6	23.3	43.1
5 HB	27.0	52.9	20.0
6 HH	–	–	–

For 3 HH, $^3J(C(3)H,C(2)H^{Re})$ is slightly smaller than $^3J(C(3)H,C(2)H^{Re})$ for all other units. Although exact populations could not be determined for this unit, the (+)-*sc* conformation can be assumed to be predominant similar to 4 HC, which has a comparably small $^3J(C(3)H,C(2)H^{Re})$. For 6 HH, the conformation about ϕ_2 could not be determined due to the degenerate chemical shift of the two $C(2)H_2$ protons.

However, since the two $C(2)H_2$ protons resonate at the same chemical shift, it can be assumed that the 6 HH unit is completely unstructured.

In both helical secondary structures proposed, the conformation about ϕ_2 is close to (−)-*sc* (−62.4° for a 2_1 and −52.1° for a 3_1 helix). In principle, either one of the two helices could, therefore, occupy a fraction of the conformational ensemble of the hexameric PHB analogue **3** of up to the product of the (−)-*sc* population of each involved residue. Excluding the totally averaged carboxy terminal 6 HH unit, the maximum possible helix content of the remaining five units of **3** is 7.5%. The HB units are similar to the repetition units in PHB. NMR Studies on linear 20mer PHB molecules [17] show that the conformational equilibrium about the angle ϕ_2 (54.6% (−)-*sc*, 35.4% *ap*, and 10% (+)-*sc*) differs from the one in the HB units (54.8% (52.9%) (−)-*sc*, 23.7% (27%) *ap*, 21.5% (20.0%) (+)-*sc* for 2 HB (5 HB)) in **3**. While the populations of the predominant (−)-*sc* conformation are similar, the *ap* conformation is significantly more populated than the (+)-*sc* conformation in the 20mer PHB as compared to the hexamer **3**, where the populations of *ap* and (+)-*sc* are almost equal. In principle, the more averaged conformation of the two HB residues may be due to the smaller size of the hexamer **3** in comparison to the 20mer PHB molecule, which makes it more flexible. However, studies on 3-HB dimers and trimers [36] indicate that the $^3J(C(3)H,C(2)H_2)$ coupling constants in this smaller compounds are comparable to those in the 20mer PHB. The conformational average of 4 HC and the two HH units differs from that in the 20mer PHB. This shows that incorporation of the bulkier side chains changes the conformation of the PHB backbone. Furthermore, the conformation of the two HB units (2 HB and 5 HB) in **3** is influenced by the presence of the modified units.

The similarity in coupling constants of the two HB units, conformational average of which about ϕ_2 differs significantly both from 4 HC and the two HH units, however, suggests that the units adopt a conformation primarily governed by their specific side chains.

Conformational Analysis: ROE Data. The overall conformation of the hexamer **3** has been analyzed by ROE distance information. All interresidual ROESY cross-peaks are given in *Table 4*, together with the relative peak intensities.

The $i,i+2$ and $i,i+3$ cross-peaks can be indicative for the 2_1 or the 3_1 helix, respectively. The shortest interresidual distance (*Table 5*) for a 3_1 helix is the $i,i+3$ $C(2)H^{Si}$,C(3)H distance of 3.2 Å; for a 2_1 helix, it is the $i,i+2$ $C(2)H^{Re}$,$C(4)H_3$ with 3.2 Å. Both distances are at least 8 Å in the respective other helix.

A total of three $i,i+2$ cross-peaks and one $i,i+3$ cross-peak have been observed. Two of the three $i,i+2$ cross-peaks involve $C(2)H_2$ protons. One is between 1 HC,$C(2)H^{Si}$ and 3 HH,$C(4)H_2$. The corresponding $C(4)H_3$,$C(2)H^{Re}$ cross-peak, which would be indicative for a 2_1 helix (*Table 5*), was not observed.

The other $i,i+2$ cross-peak has been observed between 3 HH,$C(6)H_3$ and 5 HB,$C(2)H^{Si}$. Although no information on the distance between these protons is available, it can be assumed that they are far apart in either of the helix conformations, where the bulky side chains are pointing away from the helix center. The $i,i+3$ cross-peak is not indicative for the presence of a 3_1 helix. Since none of the cross-peaks that are indicative for either of the proposed helices could be found in the ROESY spectrum, whereas other nonindicative ROEs are observed, none of the helices can be

Table 4. *Inter-residual ROEs Extracted from a ROESY Spectrum of* **3**. H,H Distances have been taken from model structures of the two proposed PHB helices. H,H Distances involving *C*(5)- and *C*(6)-side-chain protons could not be determined (n.d.). An estimate of the peak intensity is given. For the degenerate 6 HH $C(2)H_2$ protons, the distance to $C(2)H^{Re}$ as well as $C(2)H^{Si}$ is given.

Residue 1	Atom identity 1	Residue 2	Atom identity 2	Residue distance	Atom distance [Å]		ROE Intensity
					3_1	2_1	
1 HC	C(3)H	2 HB	$C(4)H_3$	$i,i+1$	6.2	5.3	weak
	$C(2)H^{Re}$	2 HB	$C(4)H_3$	$i,i+1$	4.9	5.4	weak
	$C(2)H^{Re}$	2 HB	C(3)H	$i,i+1$	4.7	4.1	weak
	$C(2)H^{Si}$	2 HB	$C(4)H_3$	$i,i+1$	5.1	5.3	strong
	C(4)H	3 HH	$C(4)H^{Down}$	$i,i+2$	>8	6.0	overlapping
	$C(2)H^{Si}$	3 HH	$C(4)H^{Down}$	$i,i+2$	>8	4.8	strong
	C(4)H	4 HC	C(4)H	$i,i+3$	6.4	>8.0	weak
	$C(2)H^{Si}$	5 HB	$C(4)H_3$	$i,i+4$	7.0	>8.0	weak
2 HB	$C(2)H^{Si}$	3 HH	$H(6)H_3$	$i,i+1$	n.d.	n.d	strong
	$C(2)H^{Si}$	3 HH	$C(4)H^{Down}$	$i,i+1$	5.1	5.3	weak
3 HH	C(3)H	4 HC	C(3)H	$i,i+1$	4.9	4.5	strong
	C(5)H	5 HB	$C(2)H^{Si}$	$i,i+2$	n.d.	n.d	overlapping
4 HC	C(3)H	5 HB	$C(4)H_3$	$i,i+1$	6.2	5.3	weak
	C(3)H	5 HB	C(3)H	$i,i+1$	4.9	4.5	weak
5 HB	C(3)H	6 HH	$C(4)H^{Down}$	$i,i+1$	6.2	5.3	weak
	C(3)H	6 HH	C(3)H	$i,i+1$	4.9	4.5	overlapping
	C(3)H	6 HH	$C(6)H_3$	$i,i+1$	n.d.	n.d.	strong
	C(3)H	6 HH	C(2)H	$i,i+1$	6.5/6.9	4.4/5.6	weak
	$C(4)H_3$	6 HH	C(3)H	$i,i+1$	6.2	6.5	weak

Table 5. *Characteristic Inter-Residual Distances in the Two Proposed Helices*

Atom identity 1	Atom identity 2	Residue distance	Atom distance [Å]	
			3_1	2_1
C(4)H	$C(2)H^{Re}$	$i,i+2$	>8.0	3.2
C(4)H	$C(2)H^{Si}$	$i,i+2$	7.0	4.8
C(3)H	$C(2)H^{Re}$	$i,i+3$	4.6	>8.0
C(3)H	$C(2)H^{Si}$	$i,i+3$	3.2	>8.0

assumed to be predominantly populated. This is in agreement with the analysis of the conformation about the angle ϕ_2 (see above).

5. Discussion and Conclusions. – The conclusion from the NMR measurement of **3** is clear-cut: in the hexameric PHB analog **3**, all repetition units undergo conformational averaging, albeit to a different extent. The predominant conformation about the angle ϕ_2 for the units 1 HC, 2 HB, and 5 HB is (−)-*sc*. This is in agreement with both proposed secondary structures. However, the units 3 HH and 4 HC are predominantly in the (+)-*sc* conformation about ϕ_2. In addition, ROE data show that *neither of the two helices is present to a detectable extent on the timescale of NMR spectroscopy.* The conformational averaging of the carboxy-terminal 6 HH unit is more pronounced than that of all the other units. In the 1 HC unit, the (−)-*sc* conformation is more populated than in the other units. This can be due to the (transient) formation of an intra-residual

H-bond between the free O-terminal OH group and the adjacent ester carbonyl O-atom, as reported earlier [32]. The conformation about the angle ϕ_2 of all the units of **3** deviates from that found in linear PHB derivatives [17], indicating that the side-chain modifications have indeed altered the backbone conformation, but our going full circle from PHB to β-peptides to PHB has not led to the discovery of a predominant secondary structure! In a series of four papers, including the present one, we have now established the extremely high conformational flexibility of the polyester backbone in PHB [17][21][37]. A preferred conformation of oligo(3-hydroxybutyrates) in homogeneous solution could be detected neither by state-of-the-art NMR spectroscopy with isotopically [17] or structurally (the present paper) labeled, nor by FRET measurements [21] with fluorescence-labeled OHBs, nor by GROMOS96 molecular-dynamics simulations of the hexamers **1** ($n=1$) and **3**[11]). At the risk of writing down a truism: there is a tremendous difference between the worlds of polyesters, like PHB **1** *without*, and polyamides, like β-peptides (*cf.* **2**) *with*, H-bonding! While the latter form well-defined, predictable, structure-dependent helices, sheets, or turns, the former have random or averaged structures under the same conditions, as studied by the same methods. The structural flexibility of PHB – along with its hydrolytic instability – may be the reason why it has not shown up in crystal structures of proteins, although it has been identified as an appendage on proteins [9][12]). The structural flexibility[13]) of PHB goes with its amphiphilic properties: it can behave like a polar or like a nonpolar compound, it can be hydrophilic and hydrophobic: it may be called a 'chemical chameleon'! Each C=O group of the polymer is a dipole (and thus a H-bond acceptor and a ligand for metal ions [38]). The higher polymers are soluble neither in polar nor nonpolar solvents, and show a peculiar solubility behavior by dissolving well only in chlorinated solvents [1][39] – the solution can function as ion-transporting bulk membrane [40]. On the other hand, OHBs can be incorporated in phospholipid bilayers – functioning as ion carriers or ionophores [5–7]. In blood serum, PHB is mostly associated with albumin [1][41], the abundant transport protein for lipophilic compounds – but also the carrier of 40% of the serum Ca^{2+} content. PHB mixes with $LiClO_4$, to form a solid, conductive electrolyte composition[14]), and it can be solubilized in THF by the addition of LiCl [11][12][43][44]. Beautiful demonstrations of the 'mixed' nature of PHB were obtained, years ago, from crystal structures of the cyclic triesters (triolides $-[O-CHR-CH_2-CO]_3-$, R=Me, Et), and of inclusion compounds, and their Na^+, K^+, and Ba^{2+} complexes (*Fig. 7*).

Returning to the structure of the PHB chain, we realize that the two helices (*Figs. 1* and *8*) have totally different features and patterns: the 3_1 helix has a resulting dipole moment with an O-terminal positive and a C-terminal negative poles, with a lipophilic surface covered by Me groups, and without the capability of complexing ions (*Fig. 8,c*). The 2_1 helix has no resulting dipole moment, both, the C=O and the C–Me bonds

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[11]) See preceding report in this issue [37].

[12]) *Reusch*; see the discussion with references in [1]. Poly[(*R*)-3-hydroxybutyryl]-conjugated proteins; *cf.* farnesyl, palmitoyl, phosphoryl, sulfatyl, glycosyl, hypusinyl derivatives of proteins (*Fig. 15* in [1]).

[13]) Adjectives like *evasive*, *elusive*, *volatile*, or *fickle* come to mind!

[14]) Poly(ethylene oxide) dissolves Li salts to form crystalline electrolytes with discrete composition. The recently published structure of these crystalline complexes [42] strongly resemble to the channel-like arrangement of triolide complexes with Na^+, K^+ , and Ba^{2+} (*Fig. 7*).

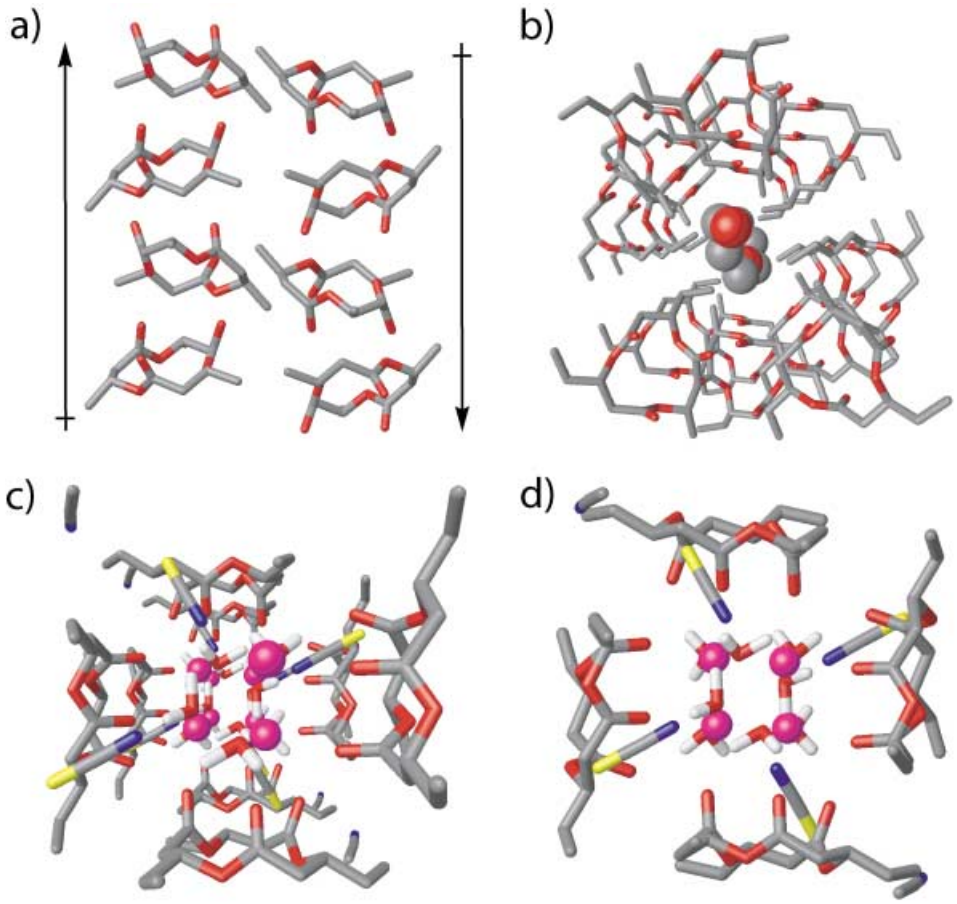

Fig. 7. *Crystal structures containing cyclic hydroxybutyrate (HB) and hydroxyvalerate (HV) trimers (triolides): models for polar and nonpolar ion channels. Grey:* C and C,C bonds; *red:* O; *blue:* N; *yellow:* S; *violet:* Na^+; *white:* H. *a*) Antiparallel stacks of HB triolide molecules (the arrows indicate the directions of the C=O dipole moments in each molecule (2.60 D [45]) and stack [14]. *b*) View along a channel filled with Et_2O molecules in the crystal of HB/HV trimers ('mixolides'); the stacks are arranged as in *a*, but, due to the presence of both Me and Et side chains, the packing of the stacks is not perfect, so that Et_2O molecules fill the gaps [15]. *c*) and *d*) Two views along a channel filled with $Na^+ \cdot 2H_2O$; the channel walls are formed by HV triolides, which turn all their C=O O-atoms inside to provide coordination sites for Na^+ and H-bond acceptor atoms for H_2O, the 'glue' between the ions and the wall; the SCN^- counter-ions stick in the wall; the composition of the crystal is HV-triolide $\cdot$ NaSCN $\cdot$ 2 H_2O $\cdot$ 0.5 MeCN [15].

point in approximately perpendicular directions with respect to the helix axis, rendering the helix surface amphiphilic and providing for ideal complexation of ions in between parallel or anti-parallel strands of helices (*Fig. 8*,*a*). The flexibility of the backbone would thus allow PHB chains to enter and be incorporated in bilayers as the 3_1 helical conformer, and switch to the 2_1 helical form under the influence of metal ions (*cf.* the proposed structures of channels, pores, and PHB $\cdot$ Ca $\cdot$ polyphospate complexes [1][6][7], and see *Figs. 7* and *8*,*b*).

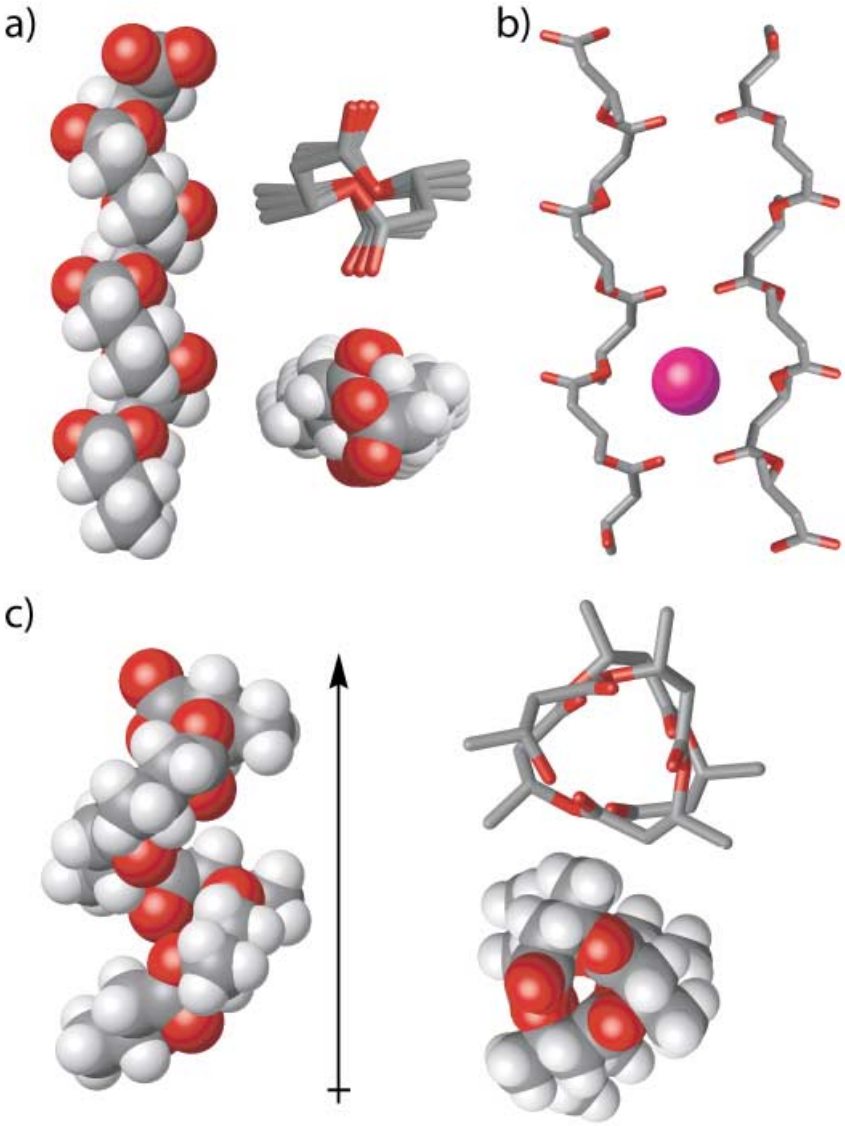

Fig. 8. *Structures and properties of the amphiphilic* 2_1 *and lipophilic* 3_1 *helices of poly[(*R*)-3-hydroxybutyrate] (PHB). Grey:* C and C,C bonds; *red:* O; *violet:* Na^+. *a*) Side view and projection along the axis of the left-handed 2_1 helix of a PHB chain [13][15]; the helix surface is amphiphilic, covered by C=O and MeC groups, which point in perpendicular directions; there is no resulting dipole moment. *b*) Two antiparallel 2_1-helical PHB columns complexing a Na^+ ion; *cf.* the proposed PHB ion-channel structures [5–7][12][15][46] and the proteinaceous K^+ channel in *Fig. 9*. *c*) Side view and projection along the right-handed 3_1 helix of a PHB chain; the helix has a large resulting dipole moment (see arrow; *cf.* polarization of phospholipid bilayers in cell walls), its surface is lipophilic, covered with Me groups [13][15].

There is a striking resemblance between the geometries of a 2_1-helical PHB ion complex, as depicted in *Fig. 8,b*, and the so-called selectivity filter of the highly preserved part of natural-protein potassium channels consisting of an array of glycine, valine, threonine, and tyrosine residues, in which four subunits provide amide C=O O-atoms in a proper arrangement for complexation and passage of the partially desolvated K ions [47] (*Fig. 9*)[15]. Recent synthetic model chemistry of the potassium channel in combination with NMR spectroscopy, indeed, suggest cooperative forming and breaking of four H-bonds in the channel to accomodate ion passage through the filter [49][16].

As *Reusch* [4][52] and *Seebach et al.* [1][2][51] have speculated before, PHB might be a primordial or ancient biopolymer, which might have taken the role of an ion-channel material in an *RNA world* without proteins, when both genetic information and enzymatic activity were provided by RNA. Phospholipids and PHB may have been congeners in this RNA world, both being formed by coupling of acetoacetic acid (3-oxobutanoic acid) units in a reducing atmosphere, PHB by simple reduction and polymerizing, and esterification, and the lipids by two reduction steps and C,C-bond formations[17]) (see, *e.g.*, the hypothetical proposals for the origin of life as forwarded by *Wächtershäuser* [54]). Structural similarities between PHB and protein channels may originate from the evolution of the latter after the design of the former.

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Experimental Part

1. *General*. The solvents used were either *puriss.*, *p. a.* quality or distilled over appropriate drying agents. FC: *Fluka* silica gel *60* (40–63 μm) at *ca.* 0.3 bar. M.p.: *Büchi 510* apparatus; not corrected. Optical rotation: *Perkin-Elmer 241* polarimeter (10 cm, 1 ml cell) at r.t. CD Spectra: *Jasco J-710*, recording from 200 to 250 nm at 20°; 1-mm rectangular cell; average of five scans, corrected for the baseline; molar ellipticity in $\deg \cdot cm^2 \cdot dmol^{-1}$ (λ in nm); smoothing by *Jasco* software. IR Spectra: *Perkin-Elmer 782* spectrophotometer. NMR: All measurements for the structual investigations have been performed on *Bruker DRX-600* spectrometers in the

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[15]) There are additional, intriguing connections between PHB and the microbial KcsA potassium channel, which consist of four protein subunits [44][47]: the crystal structure, from which the presentations in *Fig. 9* were prepared, was obtained with a protein from which 25% of the amino acid residues of the natural protein had been removed. According to investigations by *Reusch*, there is an amino acid in the removed part of the protein that bears a PHB ester chain complexing polyphosphate [4][9]. Point mutation in the removed part leads to a protein that lacks PHB, oligomerizes poorly to the tetrameric quarternary structure, and leads to a channel that does not gate like the natural system. The role of the PHB appendage in the potassium-channeling mechanism of the membrane-bound protein is unknown at this stage. We thank *R. N. Reusch* for communicating unpublished results to *D. Seebach*.

[16]) Interestingly, the *Schultz* group has just published the preparation of two (depsipeptide) KcsA mutants with an (*S*)-2-hydroxy-3-(4-hydroxyphenyl)propionate unit (the O analog of Tyr) in position 145 or an (*S*)-2-hydroxy-3-phenylpropionate unit (the O analog of Phe) in position 147 of the selectivity filter [50].

[17]) The microbial enzyme by which the high-molecular-weight storage PHB is assembled from Me–CH(OH)–CH_2–CO–S-Coenzyme A is well-known [3], and the mechanism has been studied in great detail [53]. The *Claisen* condensation of acetic acid to 3-oxobutanoic acid is common to the biosynthetic pathways leading to PHB [3] and to fatty acids (see textbooks of biochemistry and monographs on enzymatic reaction mechanisms).

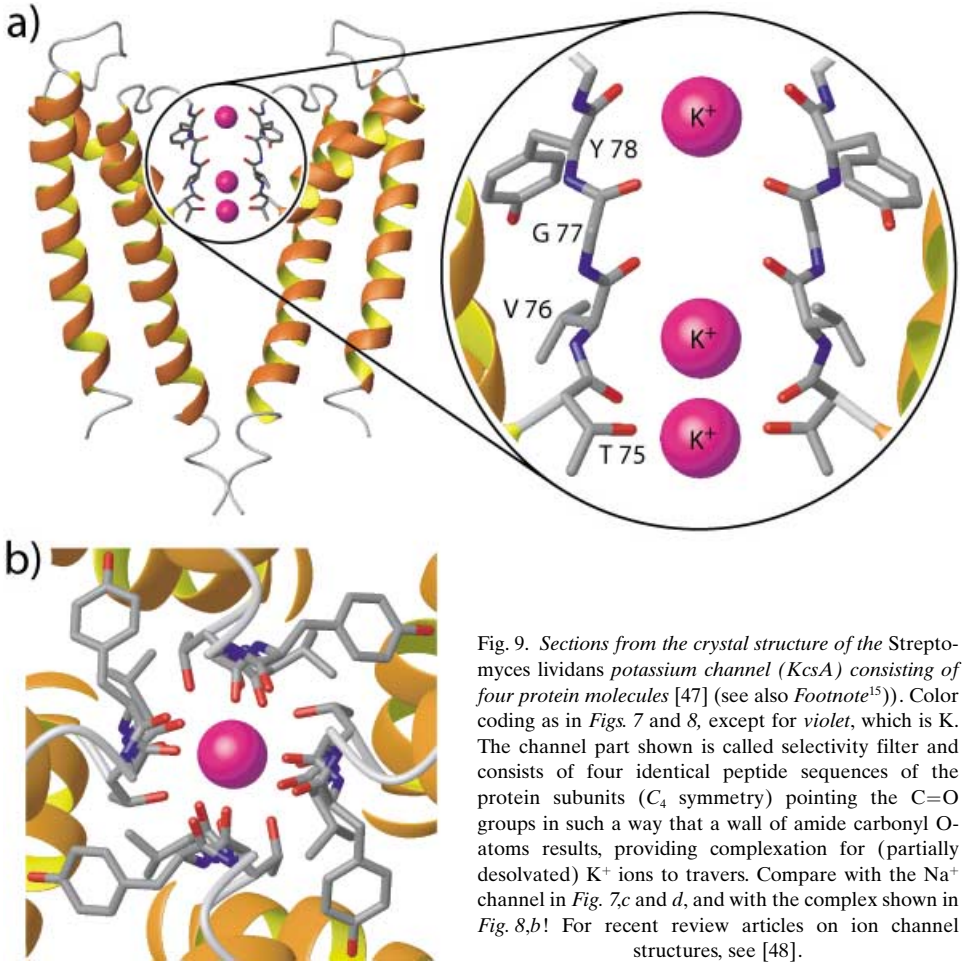

Fig. 9. *Sections from the crystal structure of the* Streptomyces lividans *potassium channel (KcsA) consisting of four protein molecules* [47] (see also *Footnote*[15])). Color coding as in *Figs. 7* and *8,* except for *violet*, which is K. The channel part shown is called selectivity filter and consists of four identical peptide sequences of the protein subunits (C_4 symmetry) pointing the C=O groups in such a way that a wall of amide carbonyl O-atoms results, providing complexation for (partially desolvated) K^+ ions to travers. Compare with the Na^+ channel in *Fig. 7,c* and *d*, and with the complex shown in *Fig. 8,b*! For recent review articles on ion channel structures, see [48].

Francis Bitter Magnet Laboratory and the Department of Chemistry at MIT (*NIH* grant 1S10RR13886-01) at 20°. The spectra have been recorded on a 10-mg sample in 500 µl $CHCl_3$; *Bruker AMX-500* (^{1}H: 500 MHz, ^{13}C: 125 MHz), *AMX-400* (^{1}H: 400 MHz, ^{13}C: 100 MHz); chemical shifts δ in ppm downfield from internal Me_4Si (=0 ppm); *J* values in Hz. MS: *Finnigan MAT TSQ*-7000 (ESI); in *m/z* (% of basis peak). Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

2. *Preparation of the Acid Chloride. General Procedure* 1 (*GP 1*). Similarly to the procedure in [28], the carboxylic acid was dissolved in CH_2Cl_2, and oxalyl chloride (1.5 equiv.), and one drop DMF were added. The mixture was stirred at r.t., until the gas evolution ceased (2–8 h). The solvent was removed *in vacuo*, and the oily residue was dried under high vacuum.

3. *Coupling of the Acid Chloride with the Corresponding Alcohol. General Procedure 2* (*GP 2*). Similarly to the procedure in [28], the well-dried acid chloride was dissolved under Ar in CH_2Cl_2, and cooled to $-78°$. After the addition of a soln. of the appropriate alcohol (1.0 equiv.) in CH_2Cl_2, a soln. of pyridine (1.0 equiv.) in CH_2Cl_2 was slowly injected. The mixture was allowed to warm to r.t. within 12 h and stirred for another 10 h. Subsequent dilution with Et_2O was followed by thorough washing with IM HCl (2×), sat. $NaHCO_3$ and sat. NaCl solns. The org. phase was dried ($MgSO_4$) and concentrated *in vacuo*.

4. *Removal of the Benzyl Ester Protecting Group. General Procedure 3* (*GP 3*). The benzyl-ester-protected oligoester was dissolved in MeOH, and a catalytic amount of 10% Pd/C was added. The apparatus was evacuated and flushed three times with H_2. After the mixture was stirred under H_2 (balloon) for 18 h, subsequent filtration through *Celite* and concentration *in vacuo* yielded the crude carboxylic acid.

5. *(t-Bu)Ph$_2$SiO Deprotection. General Procedure 4* (*GP 4*). The appropriate (*tert*-butyl)diphenylsilyl ether was dissolved in CH_2Cl_2 in a polyether flask and cooled to 0°. A soln. of 70% HF · pyridine was added, and the biphasic system was vigorously stirred for 20 min. The emulsion was poured into H_2O, Et_2O was added, and the org. phase was separated. The org. phase was washed subsequently with H_2O (2×), sat. $NaHCO_3$ (3×) and sat. NaCl. solns., dried ($MgSO_4$), and evaporated under reduced pressure.

6. *Preparation of the [Ru((*R*)-binap)Cl$_2$] Catalyst. General Procedure 5* (*GP 5*). Similarly to the procedure in [32], [Ru(cod)-(2-methylallyl)$_2$] complex and binap (=2,2′-bis(diphenylphosphanyl)-1,1′-binaphthalene) were dissolved in degassed acetone, and a soln. of HCl in MeOH was slowly added. The resulting orange soln. was stirred for 1 h, and the solvent was removed *in vacuo* to yield the dihalogeno complex, which was used directly without further purification.

Methyl 4-Methyl-3-oxopentanoate (**4**). According to the procedure in [30], a soln. of *Meldrum*'s acid (50.0 g, 0.35 mol) and pyridine (55.0 ml, 53.9 g, 0.68 mol, 1.9 equiv.) in CH_2Cl_2 (250 ml) was cooled to 0°. Isobutanoyl chloride (36.7 ml, 37.3 g, 0.35 mol) was added slowly. The orange soln. was stirred for 1 h at 0° and then at r.t. for 1 h. The mixture was subsequently washed with 1M HCl (3×) and sat. NaCl solns. The org. phase was dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The red oily crude product was refluxed 4 h in MeOH (100 ml). After removal of the solvent *in vacuo*, the residue was distilled (63°, 13 Torr) over a 50-cm *Vigreux* column. Subsequent FC (SiO_2; Pentane/ Et_2O 5:1) yielded **4** (17.0 g, 0.12 mol, 29%). Colorless oil. ^{1}H-NMR (200 MHz, $CDCl_3$): keto tautomer: 1.12 (*d*, *J* = 6.9, *Me*$_2$CH); 2.71 (*sept.*, *J* = 6.9, Me$_2$C*H*); 3.50 (*s*, CH_2); 3.72 (*s*, MeO); enol tautomer: 1.12 (*d*, *J* = 7.1, *Me*$_2$CH); 2.40 (*sept.*, *J* = 7.1, Me$_2$C*H*); 3.72 (*s*, MeO); 4.98 (*d*, *J* = 0.8, C*H* = COH).

Methyl 5-Methyl-3-oxohexanoate (**5**). A soln. of *Meldrum*'s acid (30.0 g, 0.21 mol) and pyridine (33.0 ml, 32.3 g, 0.41 mol, 1.9 equiv.) in CH_2Cl_2 (150 ml) was cooled to 0°. Isopentanoyl chloride (25.3 g, 25.6 ml, 0.21 mol) was added slowly. The orange soln. was stirred for 1 h at 0° then at r.t. for 1 h. The mixture was subsequently washed with 1M HCl (3×) and sat. NaCl solns. The org. phase was dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The red oily crude product was refluxed 4 h in MeOH (300 ml). After removal of the solvent *in vacuo*, the residue was distilled (14 Torr, 80°) over a 50-cm *Vigreux* column. Subsequent FC (SiO_2; Pentan/ Et_2O 5:1) yielded **5** (12.7 g, 0.08 mol, 39%). Colorless oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.91 (*d*, *J* = 6.6, *Me*$_2$CH); 2.02–2.20 (*m*, Me$_2$C*H*); 2.39 (*d*, 7.06, Me$_2$CHC*H*$_2$); 3.40 (*s*, COCH_2CO); 3.71 (*s*, MeO).

*Methyl (*S*)-4-Methyl-3-hydroxypentanoate* (**6**). The catalyst was prepared *in situ* according to *GP 5* with [Ru-(cod)(2-methylallyl)$_2$] complex (10.1 mg, 32 mmol), (*R*)-binap (23.5 mg, 38 mmol, 1.2 equiv.), and 0.24M methanolic HCl (0.29 ml, 69 mmol, 2.2 equiv.). A degassed soln. of **4** (5.00 g, 34.7 mmol) in MeOH (5 ml) was added to the dried catalyst. This orange suspension was immediately placed in an autoclave, which was purged with H_2 (3×) and pressurized under 100 atm. After stirring at r.t. for 72 h, the solvent was removed *in vacuo*, and the residue was distilled under reduced pressure (13 Torr, 78°) to yield **6** (4.50 g, 30.3 mmol, 89%). Colorless oil. $[\alpha]_D^{r.t.} = -43.9$ (*c* = 1.00, $CHCl_3$). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.92 (*d*, *J* = 5.8, Me–C(4)); 0.95 (*d*, *J* = 5.8, Me–C(4)); 1.71 (*oct.*, *J* = 5.8, H–C(4)); 2.40 (*dd*, *J* = 16.2, 9.1, 1 H–C(2)); 2.52 (*dd*, *J* = 16.2, 3.7, 1 H–C(2)); 2.78 (*s*, OH); 3.71 (*s*, MeO); 3.78 (*ddd*, *J* = 3.7, 5.8, 9.1, H–C(3)).

*Methyl (*R*)-5-Methyl-3-hydroxyhexanoate* (**7**). The catalyst was prepared *in situ* according to *GP 5* with [Ru-(cod)(2-methylallyl)$_2$] complex (9.2 mg, 28 mmol), (*R*)-binap (21.5 mg, 34 mmol, 1.2 equiv.) and 0.24M methanolic HCl (0.26 ml, 64 mmol, 2.2 equiv.). A degassed soln. of **5** (5.00 g, 31.6 mmol) in MeOH (5 ml) was added to the dried catalyst. This orange suspension was immediately placed in an autoclave, which was purged with H_2 (3×) and pressurized under 100 atm. After stirring at r.t. for 72 h, the solvent was removed *in vacuo*, and the residue was distilled under reduced pressure (12 Torr, 83°) to yield **7** (4.85 g, 30.3 mmol, 96%). Colorless oil. $[\alpha]_D^{r.t.} = -15.8$ (*c* = 1.00, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): 0.91 (*d*, *J* = 6.3, *Me*$_2$CH); 1.18 (*ddd*, *J* = 13.9, 8.6, 4.6, 1 H–C(4)); 1.48 (*ddd*, *J* = 13.9, 9.0, 5.6, 1 H–C(4)); 1.70–1.88 (*m*, H–C(5)); 2.39 (*dd*, *J* = 16.5, 8.2, 1 H–C(2)); 2.49 (*dd*, *J* = 16.5, 3.4, 1 H–C(2)); 2.67 (*s*, OH); 3.7 (*s*, MeO); 4.04–4.12 (*m*, H–C(3)).

*(R)-3-[(*tert*-Butyl)diphenylsilyloxy]butyric Acid* (**9**). According to the procedure in [28], a soln. of *methyl 3-hydroxybutyrate* **8** (10.0 g, 84.6 mmol) and 1*H*-imidazole (7.48 g, 109 mmol, 1.3 equiv.) in DMF (250 ml) was cooled to 0°. At this temp., TBDPS-Cl (23.2 g, 84.1 mmol; containing 5 mol-% silanol) was added and the mixture was warmed to r.t. within 2 h. After 2 h, 4-(dimethylamino)pyridine (DMAP; 10.3 g, 84.6 mmol) was added, and the solution was stirred further for 18 h. The solvent was removed under high vacuum at 40°, and the

residue diluted with Et_2O (200 ml). The org. phase was washed subsequently with 1M HCl, sat. $NaHCO_3$, and sat. NaCl solns., dried ($MgSO_4$), and evaporated *in vacuo*. The resulting crude product was dissolved in a cooled (ice bath) soln. of KOH (7.12 g, 127 mmol, 1.5 equiv.) in EtOH (500 ml), and the resulting soln. was allowed to warm to r.t. within 12 h. The solvent was evaporated, and H_2O was added. The H_2O sol. was extracted with Et_2O (2×), acidified at pH 1 with conc. HCl, and subsequently extracted with CH_2Cl_2 (3×). The CH_2Cl_2 phase was dried and evaporated. Recrystallization (hexane, 3×) yielded **9** (18.8 g, 55 mmol, 65%). White solid. M.p. 71–72°. $[\alpha]_D^{r.t.} = -5.94$ ($c = 1.00$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.07 (s, *t*-Bu); 1.15 (d, $J = 6.2$, Me); 2.51 (*d*, $J = 5.68$, H–C(2)); 4.21–4.31 (*m*, H–C(3)); 7.35–7.48 (*m*, 4 arom. H); 7.66–7.70 (*m*, 11 arom. H).

*(S)-3-[(*tert*-Butyl)diphenylsilyloxy]-4-methylpentanoic Acid* (**10**): A soln. of **6** (4.00 g, 27.4 mmol) and 1*H*-imidazole (2.41 g, 35.6 mmol, 1.3 equiv.) in DMF (250 ml) was cooled to 0° and treated with TBDPS-Cl (7.55 g, 27.4 mmol; containing 5 mol-% silanol). The mixture was stirred at 0° for 2 h and then warmed to r.t. within 2 h. After 2 h, DMAP (3.34 g, 27.4 mmol) was added, and the mixture was stirred further for 18 h. The solvent was removed under high vacuum at 40°, and the residue was diluted with Et_2O (200 ml). The org. phase was subsequently washed with 1M HCl, sat. $NaHCO_3$, and sat. NaCl solns., dried ($MgSO_4$), and evaporated under reduced pressure. The resulting crude product was dissolved in a cooled (ice bath) soln. of KOH (2.31 g, 41.1 mmol, 1.5 equiv.) in EtOH (150 ml), and the resulting soln. was allowed to warm at r.t. within 12 h. The solvent was evaporated, and H_2O was added. The aq. solution was extracted with Et_2O (2×), acidified to pH 1 with conc. HCl, and subsequently extracted with CH_2Cl_2 (3×). The CH_2Cl_2 phases were dried and evaporated. Recrystallization (hexane; 3×) yielded **10** (1.71 g, 16.7 mmol, 61%). White solid. M.p. 104–105°. $[\alpha]_D^{r.t.} = -2.0$ ($c = 1.00$, $CHCl_3$). IR ($CHCl_3$): 3072*m*, 2961*s*, 2858*m*, 2700*m*, 2623*m*, 1955*w*, 1894*w*, 1822*w*, 1709*s*, 1465*m*, 1427*m*, 1299*m*, 1206*m*, 1110*s*, 1077*s*, 1010*m*, 945*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.77 (*d*, $J = 6.8$, 1 Me–C(4)); 0.89 (*d*, $J = 6.8$, 1 H–C(4)); 1.04 (*s*, *t*-Bu); 1.73 (*dsept.*, $J = 6.9, 3.5$, H–C(4)); 2.37 (*dd*, $J = 15.4, 5.6$, 1 H–C(2)); 2.49 (*dd*, $J = 15.4, 6.9$, 1 H–C(2)); 4.03 (*ddd*, $J = 6.9, 5.6, 3.5$, H–C(3)); 7.30–7.45 (*m*, 4 arom. H); 7.62–7.70 (*m*, 11 arom. H); 10.65 (br. *s*, COOH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.3; 17.4; 19.4; 27.0; 33.3; 38.1; 74.4; 127.4; 127.5; 129.6; 129.7; 133.7; 133.9; 135.9; 136.0; 177.7. MS: 313.1 (48, $[M - (t\text{-Bu})]^+$); 295.1 (2), 271.3 (12), 241.1 (2), 235.1 (10), 199.1 (100), 193.1 (5), 181.1 (5), 157.1 (2), 139.0 (18). Anal. calc. for $C_{22}H_{30}O_3Si$ (370.56): C 71.31, H 8.16; found C 71.13, H 8.03.

*(*R*)-3-Hydroxy-5-methylhexanoic Acid* (**11**). The methyl ester **7** (4.00 g, 25 mmol) was dissolved in a cooled (ice bath) soln. of KOH (2.0 g, 38 mmol, 1.5 equiv.) in EtOH (100 ml). The resulting soln. was allowed to warm to r.t. within 12 h, and stirred further for 12 h at the same temp. The solvent was evaporated, and H_2O (50 ml) was added. The aq. soln. was then extracted with Et_2O, acidified with conc. HCl to pH 1, and extracted with CH_2Cl_2 (3×). The CH_2Cl_2 phase was dried ($MgSO_4$) and evaporated. Recrystallization (hexane; 2×) yielded **11** (2.69 g, 18.4 mmol, 74%). Colorless needles. M.p. 64°. $[\alpha]_D^{r.t.}$ −14.2 ($c = 1.00$, $CHCl_3$) [55]: $[\alpha]_D^{r.t.}$(*ent*-**11**) = +14.2, ($c = 1.00$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.94 (*d*, $J = 6.5$, Me_2CH); 1.18 (*ddd*, $J = 13.7, 8.7, 4.7$, 1 H–C(4)); 1.48 (*ddd*, $J = 13.7, 8.7, 5.6$, 1 H–C(4)); 1.73–1.87 (*m*, H–C(5)); 2.44 (*dd*, $J = 16.6, 8.7$, H–C(2)); 2.53 (*dd*, $J = 16.6, 3.4$, H–C(2)); 4.08–4.17 (*m*, H–C(3)). EI-MS: 145.1 (<1, $[M - H]^+$), 129.0 (2), 113.0 (3), 89.0 (100), 71.0 (81). Anal. calc. for $C_7H_{14}O_3$ (146.18): C 57.51, H 9.65; found C 57.55, H 9.73.

*Benzyl (*R*)-3-Hydroxy-5-methylhexanoate* (**12**). Compound **11** (5.00 g, 34 mmol) was dissolved in a suspension of $NaHCO_3$ (10.9 g, 102 mmol, 3.0 equiv.) in DMF (100 ml). BnBr (6.4 g, 4.5 ml, 38 mmol, 1.1 equiv.) was added, and the suspension was stirred for 24 h at r.t. After the addition of H_2O (500 ml), the soln. was extracted with Et_2O (4×). The org. phase was washed with sat. NaCl soln. and evaporated under reduced pressure. FC (pentane/Et_2O 5:1) yielded **12** (7.51 g, 31.8 mmol, 93%). Colorless oil. $[\alpha]_D^{r.t.} = -15.8$ ($c = 1.00$, $CHCl_3$). IR ($CHCl_3$): 3585*w* (br.), 3007*m*, 2959*m*, 2871*w*, 1721*s*, 1497*w*, 1455*w*, 1405*w*, 1385*m*, 1317*m*, 1270*m*, 1170*s*, 1072*w*, 1041*w*, 970*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.91 (*d*, $J = 6.7$, Me_2CH); 1.18 (*ddd*, $J = 13.8, 8.6, 4.4$, 1 H–C(4)); 1.48 (*ddd*, $J = 13.8, 8.9, 5.5$, 1 H–C(4)); 1.76–1.84 (*m*, H–C(5)); 2.44 (*dd*, $J = 16.5, 8.8$, 1 H–C(2)); 2.53 (*dd*, $J = 16.5, 3.3$, 1 H–C(2)); 2.87 (*d*, $J = 4.0$, OH); 4.07–4.14 (*m*, H–C(3)); 5.15 (*s*, PhCH_2); 7.29–7.44 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.0; 23.3; 24.4; 41.8; 45.6; 66.1; 66.5; 128.3; 128.4; 128.6; 135.6; 172.8. EI-MS: 236.1 (4, M^+), 190.1 (4), 179.1 (5), 150.0 (<1), 127.1 (2), 107.0 (44), 91.0 (100).

*Benzyl (3*R*)-3-([[(3′*R*)-3′-(*tert*-Butyl)diphenylsilyloxy]butanoyl}oxy)-5-methylhexanoate* (**13**). Preparation of the acid chloride according to *GP 1* with **9** (5.01 g, 14.6 mmol) and oxalyl chloride (1.88 ml, 2.78 g, 21.9 mmol, 1.5 equiv.) in CH_2Cl_2 (50 ml). The acid chloride was subsequently coupled, according to *GP 2,* at −78° with **12** (3.44 g, 14.6 mmol) in CH_2Cl_2 (50 ml), in the presence of pyridine (1.73 g, 1.76 ml, 1.5 equiv.). FC (pentane/Et_2O 7:1) yielded **13** (6.3 g, 11.3 mmol, 77%). Colorless oil. $[\alpha]_D^{r.t.} = +7.9$ ($c = 1.00$, $CHCl_3$). IR ($CHCl_3$): 3007*w*, 2964*m*, 2930*m*, 2858*m*, 1964*w*, 1897*w*, 1820*w*, 1732*s*, 1471*w*, 1427*m*, 1381*m*, 1304*m*, 1172*m*, 1111*s*, 997*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.84 (*d*, $J = 6.5$, Me–C(5)); 0.86 (*d*, $J = 6.4$, Me–C(5)); 1.03 (*s*, *t*-Bu); 1.11 (*d*, $J = 6.1$, 3 H–C(4′)); 1.25–1.32 (*m*, H–C(5)); 1.48–1.59 (*m*, 2 H–C(4)); 2.34 (*dd*, $J = 14.7, 7.4$, 1 H, CH_2);

2.48 (*dd*, *J* = 14.7, 5.3, 1 H, CH_2); 2.51 (*dd*, *J* = 15.3, 6.0, 1 H, CH_2); 2.58 (*dd*, *J* = 15.3, 6.7, 1 H, CH_2); 4.20–4.27 (*m*, H–C(3′)); 5.04 (*d*, *J* = 12.3, 1 H, $PhCH_2$); 5.08 (*d*, *J* = 12.3, 1 H, $PhCH_2$); 5.21–5.27 (*m*, H–C(3)); 7.27–7.43 (*m*, 11 arom. H); 7.65–7.68 (*m*, 4 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 19.2; 22.0; 22.9; 23.3; 24.5; 26.9; 39.6; 43.0; 44.5; 66.4; 68.9; 127.5; 127.6; 128.3; 128.4; 128.5; 129.6; 129.7; 134.0; 134.3; 135.7; 135.8; 135.9; 170.1; 170.5. EI-MS: 559.3 (4, $[M-H]^+$), 503.2 (100, $[M-C_4H_9]^+$); 483.3 (20, $[M-C_6H_5]^+$), 375.1 (8), 285.1 (70), 265.1 (44), 239.1 (10), 219.2 (19), 207.1 (24), 199.1 (47), 197.1 (21), 139.0 (25), 137.0 (22), 135.0 (45). Anal. calc. for $C_{34}H_{44}O_5Si$ (560.81): C 72.82, H 7.91; found C 73.00, H 8.16.

*Benzyl (3*R*)-3-{[(3′*R*)-3′-Hydroxybutanoyl]oxy}-5-methylhexanoate* (**14**). According to *GP 4*, **13** (1.95 g, 3.47 mmol) was treated with 70% HF · pyridine (1.44 ml, 50 mmol, 13 equiv.) in CH_2Cl_2 (25 ml) for 20 min. FC (pentane/ Et_2O 1:1) yielded **14** (1.06 g, 3.28 mmol, 95%). Colorless oil. $[\alpha]_D^{r.t.} = -11.3$ (*c* = 1.00 , $CHCl_3$). IR ($CHCl_3$): 3540*w*, 3007*w*, 2961*m*, 2871*w*, 1731*s*, 1455*w*, 1390*w*, 1312*w*, 1266*m*, 1172*m*, 1123*w*, 1053*w*, 975*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90 (*d*, *J* = 6.5, Me–C(5)); 0.91 (*d*, *J* = 6.4, Me–C(5)); 1.20 (*d*, *J* = 6.3, 3 H–C(4′)); 1.31–1.40 (*m*, H–C(5)); 1.52–1.67 (*m*, 2 H–C(4)); 2.32 (*dd*, *J* = 15.9, 8.8, 1 H, CH_2); 2.41 (*dd*, *J* = 15.9, 3.3, 1 H, CH_2); 2.59 (*dd*, *J* = 15.4, 5.8, 1 H, CH_2); 2.63 (*dd*, *J* = 15.4, 6.8, 1 H, CH_2); 3.06 (*d*, *J* = 3.6, OH); 4.11–4.19 (*m*, H–C(3′)); 5.10 (*d*, *J* = 12.3, 1 H, $PhCH_2$); 5.12 (*d*, *J* = 12.3, 1 H, $PhCH_2$); 5.33–5.40 (*m*, H–C(3)); 7.37–7.41 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.1; 22.5; 22.9; 24.6; 39.7; 43.1; 64.4; 66.7; 69.4; 128.4; 128.5; 128.6; 135.6; 170.4; 172.3. FAB$^+$-MS: 323.2 (100, $[M+H]^+$), 219.2 (12), 195.1 (6). Anal. calc. for $C_{18}H_{26}O_5$ (322.40): C 67.06, H 8.13; found C 66.93, H 7.93.

*Benzyl (3*R*)-3-{[(3′*R*)-3′-({(3″*S*)-3″-[(*tert*-Butyl)diphenylsilyloxy]-4″-methylpentanoyl}oxy)butanoyl]-oxy}-5-methylhexanoate* (**15**): Preparation of the acid chloride according to *GP 1* with **10** (644 mg, 1.74 mmol) and oxalyl chloride (0.22 ml, 331 mg, 2.61 mmol, 1.5 equiv.) in CH_2Cl_2 (50 ml). The acid chloride was subsequently coupled according to *GP 2* at −78° with **14** (556 mg, 1.74 mmol) in CH_2Cl_2 (50 ml), in the presence of pyridine (206 mg, 223 ml, 2.64 mmol, 1.5 equiv.). FC (pentane/Et_2O 5:1) yielded **15** (1.05 g, 1.55 mmol, 89%). Colorless oil. $[\alpha]_D^{r.t.} = +4.25$ (*c* = 1.00, $CHCl_3$). IR ($CHCl_3$): 3015*w*, 2961*m*, 2861*w*, 1734*s*, 1470*w*, 1427*w*, 1388*w*, 1282*m*, 1172*m*, 1111*m*, 1059*m*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.78 (*d*, *J* = 6.9, Me–C(4″)); 0.86 (*d*, *J* = 6.5, Me–C(5)); 0.87 (*d*, *J* = 6.9, Me–C(4″)); 0.88 (*d*, *J* = 6.5, Me–C(5)); 1.04 (s, *t*-Bu); 1.11 (*d*, *J* = 6.2, 3 H–C(4′)); 1.29–1.35 (*m*, H–C(5)); 1.52–1.60 (*m*, 2 H–C(4)); 1.69 (*dsept.*, *J* = 6.9, 3.3, H–C(4″)); 2.29 (*dd*, *J* = 15.3, 7.0, 1 H–C(2′)); 2.29 (*d*, *J* = 6.4, 2 H–C(2″)); 2.46 (*dd*, *J* = 15.3, 6.2, 1 H–C(2′)); 2.53 (*dd*, *J* = 15.4, 5.7, 1 H–C(2)); 2.59 (*dd*, *J* = 15.4, 7.1, 1 H–C(2)); 4.08 (*dt*, *J* = 6.4, 3.3, H–C(3″)); 5.04 (*dsept.*, 7.0, 6.2, H–C(3′)); 5.08 (*d*, *J* = 12.2, 1 H, $PhCH_2$); 5.11 (*d*, *J* = 12.2, 1 H, $PhCH_2$); 5.27–5.32 (*m*, H–C(3)); 7.34–7.43 (*m*, 11 arom. H); 7.62–7.70 (*m*, 4 arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$): 17.0; 17.8; 19.4; 19.5; 22.0; 23.0; 24.6; 27.0; 33.2; 39.1; 39.6; 40.7; 43.0; 66.5; 67.2; 69.3; 74.4; 127.4; 127.5; 128.3; 128.5; 128.6; 129.5; 129.6; 133.9; 134.4; 135.7; 135.9; 136.0; 169.4; 170.0; 170.7. FAB$^+$-MS: 697.5 (2, $[M+Na]^+$), 673.5 (4, $[M-H]^+$), 617.4 (100, $[M-C_4H_9]^+$), 597.5 (26), 313.2 (42), 311.2 (17), 309.2 (11), 293.2 (15), 225.1 (16), 199.1 (54), 197.2 (28), 183.1 (14), 139.0 (11), 137.1 (15), 135.1 (33). Anal. calc. for $C_{40}H_{54}O_7Si$ (674.95): C 71.18, H 8.06; found: C 71.29, H 8.21.

*(3*R*)-3-{[(3′*R*)-3′-({(3″*S*)-3″-[(*tert*-Butyl)diphenylsilyloxy]-4″-methylpentanoyl}oxy)butanoyl]oxy}-5-methylhexanoic Acid* (**16**): Treatment of a soln. of **15** (480 mg, 0.71 mmol) in MeOH (10 ml) with Pd/C (100 mg) for 28 h according to *GP 3* yielded **16** (350 mg, 0.59 mmol, 83%). Pure colorless viscous oil. $[\alpha]_D^{r.t.} = +0.3$ (*c* = 1.00, $CHCl_3$). IR ($CHCl_3$): 2961*m*, 2861*w*, 1732*s*, 1600*w*, 1471*w*, 1427*w*, 1387*w*, 1301*w*, 1262*w*, 1177*m*, 1111*m*, 1059*m*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.77 (*d*, *J* = 6.9, $CHCH_3CH_3$); 0.88 (*d*, *J* = 6.9, $CHCH_3CH_3$); 0.89 (*d*, *J* = 6.2, 3 H, Me_2CH); 0.90 (*d*, *J* = 7.3, 3 H, Me_2CH); 1.04 (*s*, *t*-Bu); 1.13 (*d*, *J* = 6.3, Me–C(3′)); 1.33–1.38 (*m*, H–C(5)); 1.55–1.60 (*m*, 2 H–C(4)); 1.70 (*dsept.*, *J* = 6.9, 3.3, H–C(4″)); 2.34–2.58 (*m*, 3 CH_2); 4.06–4.09 (*m*, H–C(3″)); 5.03–5.10 (*m*, H–C(3′)); 5.28 (br. *m*, H–C(3)); 7.34–7.44 (*m*, 6 arom. H); 7.63–7.80 (*m*, 4 arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$): 17.1; 17.7; 19.4; 19.5; 22.0; 23.0; 24.6; 27.1; 33.2; 38.9; 39.6; 40.9; 43.1; 67.4; 69.4; 74.5; 127.4; 127.5; 129.5; 129.6; 133.9; 134.3; 135.9; 136.0; 169.7; 170.9; 175.0. FAB$^+$-MS: 1025.2 (15, $[M+Na-H]^+$), 1003.2 (3, $[M+H]^+$), 945.1 (100), 925.2 (36), 419.0 (26), 352.9 (26), 329.0 (23), 312.9 (37), 311.0 (53), 308.9 (18), 225.0 (39), 219.0 (24), 215.0 (19), 201.0 (25), 197.0 (42), 183.0 (27). Anal. calc. for $C_{57}H_{82}O_{13}Si$ (1003.35): C 68.23, H 8.24; found: C 68.40, H 8.04.

*Benzyl (3*R*)-3-{[((3′*R*)-3′-{[(3″*S*)-3″-Hydroxy-4″-methylpentanoyl]oxy}butanoyl)oxy]-5-methylhexanoate* (**17**). According to *GP 4*, **15** (1.10 g, 1.62 mmol) was treated with 70% HF · pyridine (0.62 ml, 21.1 mmol, 13 equiv.) in CH_2Cl_2 (15 ml) for 20 min. FC (pentane/ Et_2O 3:1) yielded **17** (612 mg, 1.40 mmol, 87%). Colorless oil. $[\alpha]_D^{r.t.} = -10.1$ (*c* = 1.00, $CHCl_3$). IR ($CHCl_3$): 3538*w*, 3025*w*, 2962*m*, 2871*w*, 1733*s*, 1497*w*, 1467*w*, 1384*m*, 1307*m*, 1266*m*, 1175*m*, 1139*m*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.89 (*d*, *J* = 6.5, Me–C(5)); 0.90 (*d*, *J* = 6.0, Me–C(5)); 0.92 (*d*, *J* = 6.7, Me–C(4″)); 0.94 (*d*, *J* = 6.8, Me–C(4″)); 1.28 (*d*, *J* = 6.3, 3 H–C(4′)); 1.31–1.41 (*m*, H–C(5)); 1.52–1.60 (*m*, 2 H–C(4)); 1.70 (*dsept.*, *J* = 5.7, 6.8, H–C(4″)); 2.36 (*dd*, *J* = 15.9, 9.7, H–C(2″)); 2.43 (*dd*, *J* = 15.5, 5.4, 1 H–C(2′)); 2.44 (*dd*, *J* = 15.9, 2.7, H–C(2″)); 2.55 (*dd*, *J* = 15.5, 7.7, 1 H–C(2′)); 2.58 (*dd*, *J* =

15.4, 5.5, 1 H–C(2)); 2.62 (*dd*, *J* = 15.3, 7.2, 1 H–C(2)); 2.95 (*d*, *J* = 3.9, OH); 3.76 (*dddd*, *J* = 9.7, 5.7, 3.9, 2.7, H–C(3″)); 5.10 (*d*, *J* = 12.2, 1 H, PhCH_2); 5.13 (*d*, *J* = 12.2, 1 H, PhCH_2); 5.27 (*ddq*, *J* = 7.7, 6.3, 5.4, H–C(3′)); 5.30–5.35 (*m*, H–C(3)); 7.30–7.38 (*m*, 5 arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$): 17.7; 18.3; 19.8; 22.1; 22.9; 24.6; 33.2; 38.8; 39.6; 40.8; 43.1; 66.6; 67.6; 69.5; 72.7; 128.3; 128.5; 128.6; 135.7; 169.6; 170.1; 172.7. FAB$^+$-MS: 458.9 (8, $[M+Na]^+$), 437.0 (100, $[M+H]^+$), 329.0 (8), 323.0 (41), 305.0 (5), 233.0 (4), 219.0 (24), 215.0 (9), 201.0 (19), 128.9 (11), 114.9 (23). Anal. calc. for $C_{34}H_{44}O_5Si$ (436.55): C 66.03, H 8.31; found: C 65.82, H 8.19.

*Benzyl (3*R*)-3-{[(3′*R*)-3′-({(3″*S*)-3″-[((3‴*R*)-3‴-{[(3⁗*R*)-3⁗-({(3⁗′*S*)-3⁗′-[(*tert*-butyl)diphenylsilyloxy]-4⁗′-methylpentanoyl}oxy)butanoyl]oxy}-5‴-methylhexanoyl)oxy]-4″-methylpentanoyl}oxy)butanoyl]oxy}-5-methylhexanoate* (**18**). Preparation of the acid chloride according to *GP 1* with **16** (475 mg, 0.81 mmol) and oxalyl chloride (0.10 ml, 154 mg, 1.21 mmol, 1.5 equiv.) in CH_2Cl_2 (30 ml). The acid chloride was subsequently coupled according to *GP 2* at −78° with **17** (354 mg, 0.81 mmol) in CH_2Cl_2 (50 ml), in the presence of pyridine (96 mg, 98 ml, 1.21 mmol, 1.5 equiv.). FC (pentane/Et_2O 3:1) yielded **18** (520 mg, 0.51 mmol, 64%). Colorless oil. $[\alpha]_D^{rt.} = +4.5$ (*c* = 1.00, $CHCl_3$). IR ($CHCl_3$): 3037*w*, 2961*m*, 2871*w*, 1736*s*, 1600*w*, 1467*w*, 1427*w*, 1384*w*, 1307*w*, 1172*m*, 1104*m*, 1060*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.78 (*d*, *J* = 6.9, 3 H, *Me*$_2$CH); 0.87–0.90 (*m*, 21 H, *Me*$_2$CH); 1.04 (*s*, *t*-Bu); 1.14 (*d*, *J* = 6.3, *Me*CH); 1.23 (*d*, *J* = 6.3, *Me*CH); 1.30–1.38 (*m*, Me$_2$C*H*); 1.52–1.60 (*m*, 2 CHCH_2CH); 1.69 (*dsept.*, *J* = 6.9, 3.3, Me$_2$C*H*CH); 1.85–1.91 (*m*, Me$_2$C*H*); 2.34–2.64 (*m*, 6 CH_2); 4.07 (*dt*, *J* = 3.3, 6.4, H–C(3⁗′)); 5.03–5.08 (*m*, CH_2C*H*); 5.09 (*d*, *J* = 12.2, 1 H, PhCH_2); 5.12 (*d*, *J* = 12.2,1 H, PhCH_2); 5.08–5.14 (*m*, CH_2C*H*); 5.16–5.23 (*m*, CH_2C*H*); 5.23–5.28 (*m*, CH_2C*H*CH_2); 5.23–5.28 (*m*, CH_2C*H*CH_2); 7.30–7.47 (*m*, 11 arom. H); 7.63–7.70 (*m*, 4 arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$): 17.0; 17.8; 19.4; 19.5; 22.0; 23.0; 24.6; 27.0; 33.2; 39.1; 39.6; 40.7; 43.0; 66.5; 67.2; 69.3; 74.4; 127.4; 127.5; 128.3; 128.5; 128.6; 129.5; 129.6; 133.9; 134.4; 135.7; 135.9; 136.0; 169.4; 170.0; 170.7. FAB$^+$-MS: 1025.2 (15, $[M+Na]^+$), 1003.2 (3, M^+), 945.1 (100), 925.2 (36), 419.0 (26), 352.9 (26), 329.0 (23), 312.9 (37), 311.0 (53), 308.9 (18), 225.0 (39), 219.0 (24), 215.0 (19), 202.0 (25), 197.0 (42), 183.0 (27). Anal. calc. for $C_{57}H_{82}O_{13}Si$ (1003.36): C 68.23, H 8.24; found: C 68.40, H 8.04.

*Benzyl (3*R*)-3-{[(3′*R*)-3′-{[(3″*S*)-3″-{[(3*R‴*)-3‴-{[(3⁗*R*)-3⁗-{[(3⁗′*S*)-3⁗′-Hydroxy-4⁗′-methylpentanoyl]oxy}butanoyl]oxy}-5‴-methylhexanoyl]oxy}-4″-methylpentanoyl]oxy}butanoyl]oxy}-5-methylhexanoate* (**19**). According to *GP 4*, **18** (400 mg, 0.398 mmol) was treated with 70% HF·pyridine (0.13 ml, 5.17 mmol, 13 equiv.) in CH_2Cl_2 (15 ml) for 20 min. FC (pentane/ Et_2O 2:1) yielded **19** (289 mg, 378 mmol, 95%). Colorless oil. $[\alpha]_D^{rt.} = -3.0$ (*c* = 1.00, $CHCl_3$). IR ($CHCl_3$): 3538*w*, 3025*w*, 2962*m*, 2871*w*, 1736*s*, 1600*w*, 1467*w*, 1384*w*, 1307*m*, 1285*m*, 1261*m*, 1176*m*, 1139*m*, 1056*m*, 1003*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.87–0.95 (*m*, 4 *Me*$_2$CH); 1.24 (*d*, *J* = 6.3, *Me*CH); 1.31 (*d*, *J* = 6.3, *Me*CH); 1.33–1.44 (*m*, 2 *Me*$_2$CH); 1.53–1.61 (*m*, 2 CHCH_2CH); 1.66–1.75 (*m*, Me$_2$CHC*H*); 1.86–1.92 (*m*, Me$_2$C*H*); 2.36 (*dd*, *J* = 15.9, 9.7, 1 H, CH_2); 2.44 (*dd*, *J* = 15.9, 2.7, 1 H, CH_2); 2.39–2.65 (*m*, 5 CH_2); 3.00 (*d*, *J* = 3.9, OH); 3.74–3.78 (*m*, H–C(3⁗′)); 5.09 (*d*, *J* = 12.2, 1 H, PhCH_2); 5.12 (*d*, *J* = 12.2, 1 H, PhCH_2); 5.12–5.14 (*m*, MeC*H*CH_2); 5.17–5.23 (*m*, MeC*H*CH_2); 5.27–5.34 (*m*, H–C(3‴), H–C(3″), H–C(3′)); 7.31–7.47 (*m*, 5 arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$): 17.5; 17.7; 18.1; 18.4; 19.6; 19.9; 22.0; 22.1; 23.0; 24.5; 24.6; 31.1; 33.2; 36.5; 38.9; 39.5; 39.6; 40.7; 40.8; 43.0; 43.1; 66.5; 67.6; 67.7; 69.4; 69.5; 72.7; 74.7; 128.3; 128.5; 128.6; 135.7; 169.4; 169.5; 169.5; 168.7; 170.0; 172.7. FAB$^+$-MS: 788.0 (20, $[M+Na]^+$), 766.0 (100, M^+), 652.1 (4), 547.8 (3), 419.6 (6), 329.4 (4), 225.3 (3), 219.3 (5), 215.3 (3), 201.3 (7), 183.3 (4). Anal. calc. for $C_{41}H_{64}O_{13}$ (764.95): C 64.38, H 8.43; found: C 64.40, H 8.28.

*(3*R*)-3-{[(3′*R*)-3′-{[(3″*S*)-3″-{[(3*R‴*)-3‴-{[(3⁗*R*)-3⁗-{[(3⁗′*S*)-3⁗′-Hydroxy-4⁗′-methylpentanoyl]oxy}-butanoyl]oxy}-5‴-methylhexanoyl]oxy}-4″-methylpentanoyl]oxy}butanoyl]oxy}-5-methylhexanoic Acid* (**3**). Treatment of a soln. of **19** (50 mg, 66 mmol) in MeOH (4 ml) with Pd/C (12 mg) for 28 h according to *GP 3* yielded **3** (45 mg, 64 mmol, 97%). Colorless viscous oil. $[\alpha]_D^{rt.} = -9.64$ (*c* = 0.98, $CHCl_3$). IR ($CHCl_3$): 3692*w*, 3517*w*, 3025*w*, 2963*m*, 2871*w*, 1737*s*, 1605*w*, 1467*w*, 1387*w*, 1307*m*, 1261*m*, 1101*m*, 1057*w*, 1004*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.90–0.95 (*m*, 4 *Me*$_2$CH); 1.27 (*d*, *J* = 6.3, *Me*CH); 1.31 (*d*, *J* = 6.3, *Me*CH); 1.34–1.44 (*m*, 2 Me$_2$C*H*); 1.55–1.63 (*m*, 2 CHCH_2CH); 1.67–1.75 (*m*, Me$_2$C*H*CH); 1.88–1.95 (*m*, Me$_2$C*H*); 2.35–2.66 (*m*, 6 CH_2); 3.78–3.82 (*m*, H–C(3⁗′)); 4.60 (br. *s*, COOH, OH); 5.09–5.13 (*m*, CH); 5.21–5.27 (*m*, CH); 5.28–5.36 (*m*, 3 CH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.3; 17.7; 18.1; 18.3; 19.7; 19.9; 22.1; 22.2; 22.9; 22.9; 24.6; 24.7; 31.2; 3.2; 36.6; 38.8; 39.5; 39.7; 40.8; 43.0; 43.3; 67.7; 67.8; 69.6; 69.7; 72.9; 75.1; 169.6; 169.8; 169.9; 170.0; 172.8; 173.2. FAB$^+$-MS: 1416.6 (1), 719.4 (24), 697.4 (100, $[M+Na]^+$); 675.4 (4, $[M+H]^+$), 653.3 (2), 565.3 (2), 479.3 (2), 351.3 (4), 329.3 (4), 215.2 (4), 201.2 (4). Anal. calc. for $C_{34}H_{58}O_{13}$ (674.82): C 60.52, H 8.66; found: C 60.41, H 8.79.

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