Synthesis of Amino-Acid-, Carbohydrate-, Coumarin-, and Biotin-Labelled Oligo[(*R*)-3-Hydroxybutanoic Acids] (OHB)

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The benzyl esters **1**–**3** of oligo[(*R*)-3-hydroxybutanoic acids] (OHB) containing 2, 16, or 32 HB units were coupled at the hydroxy terminus with arginine (by esterification with carbodiimide), with glucose (by acetalization with glucosyl trichloroacetimidate), and with 7-(dimethylamino)coumarin-4-acetic acid and biotin (by amide formation through a glycine linker) to give, after deprotection(s), the corresponding 'labelled' OHB acids **7**–**9**, **12**, **13**, **25**, **26**, **33**, and **34** (*Schemes 1*, *4*, and *5*). The respective novel 16- and 32mer derivatives exhibit distinct water solubility (*Table*) or may be detected (in minute amounts) by fluorescence spectroscopy, properties required for biochemical investigations.

Introduction. – Poly[(*R*)-3-hydroxybutanoic acid] (PHB) and related poly(3-hydroxyalkanoates) (PHAs) are an ubiquitous class of biopolymers [1][2] occurring as high-molecular-weight storage material (sPHB) in microorganisms [3] as well as in a low-molecular-weight form (cPHB). cPHB has been detected in eucaryotic and procaryotic cells, in human aorta tissue, and in blood plasma and has been further characterized by ^{1}H-NMR spectroscopy [4][5]. Apart from the well-established ionophoric activity, the nature and function of cPHB as an integral cell constituent is still unknown. *Reusch* and coworkers have shown that cPHB (*ca.* 150 units) and $CaPP_i$ (calcium polyphosphate, *ca.* 75 units) form a complex which may function as an ion channel in genetically competent *Escherichia coli* [5]. The ionophoric ability of this non-proteinaceous calcium-selective channel was unambiguously demonstrated in recent patch-clamp experiments, by comparison of the complex extracted from *E. coli* membrane with a complex that was constituted from a synthetic oligo[(*R*)-3-hydroxybutanoate] (OHB)[2]), containing 128 HB units, and inorganic polyphosphate [6]. Furthermore, the cation-transport activity of OHBs themselves has been amply demonstrated: they are able to transport cations across CH_2Cl_2 layers ('bulk membranes') in U-tubes [7], and they generate high-conductivity nonselective ion channels as shown in patch-clamp experiments in lipid bilayers [8]. The ion-transport activity of the 16mer and of longer-chain OHBs has been attributed to the formation of hydrophobic aggregates in the lipid bilayer [8]; and these aggregates may have structures related to the lamellar crystallites of *ca.* 50-Å thickness, characteristic of OHB and PHB, in which the chains are arranged as 2_1-helix strings (pitch: 6 Å) consisting of 16 HB units [9].

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[1]) Part of the projected Ph.D. Thesis of *M.G.F.*, ETH-Zürich, 1998.

[2]) The term oligomer refers to monodisperse compounds. Material with a molecular-weight distribution is designated as polymer.

One drawback for the use of OHBs in transport studies turned out to be their peculiar solubility properties and the difficulty of their (quantitative) detection. Short-chain OHBs ($\leq$ 8 units) are soluble in common organic solvents as well as in H_2O[3]). In contrast, long-chain OHBs are neither soluble in H_2O nor in common polar or apolar media, with the exception of polyhalogenated solvents (*cf.* CH_2Cl_2, $CHCl_3$, $ClCH_2CH_2Cl$, CF_3CH_2OH). Long-chain OHBs tend to form insoluble aggregates[4]) in H_2O. The fact that spontaneous incorporation of OHBs into lipid bilayers (planar bilayers, vesicles, or cell membranes) does not occur might be a consequence of their poor water solubility and lack of amphiphilic character, *i.e.*, that they have low bioavailability. The detection of sPHB and cPHB in biological systems is usually achieved by an antibody test [5] or by a destructive analysis (heating in conc. H_2SO_4 solution to generate crotonic acid, subsequently determined by HPLC) [11][12][5]). Thus, a non-destructive method to localize and to quantify OHB material in minute amounts had to be developed.

In the present paper, we describe the synthesis of OHB derivatives with enhanced water solubility, achieved by covalent coupling with an amino-acid or a carbohydrate moiety. Furthermore, we present the synthesis of fluorescence-labelled OHBs as well as of biotin-OHB conjugates which can be easily detected by an immunoassay with fluorescence-labelled avidin [13]. The new compounds thus available were intended to be used for ion-transport studies with liposomes [14].

Syntheses and Conclusion. – *Preamble.* Monodisperse OHBs **1**–**3** were synthesized by a segment-coupling strategy previously developed by us [15] (*Scheme 1*). The subsequent derivatization at the hydroxy and not at the carboxy terminus of the OHBs was chosen for two reasons: *i*) previous patch-clamp experiments had indicated the necessicity of a free carboxylate function for successful incorporation and stability of OHBs in planar lipid membranes [8], and *ii*) OHBs tend to fragment *via* β-elimination under basic conditions [15] (following a mechanism in which an OHB-species activated at the carboxy terminus turned out to be a key intermediate). Thus, derivatization involving the carboxy functionality would have been limited to reaction conditions used in the segment coupling synthesis (*via* an acid chloride), whereas coupling at the hydroxy end should allow the use of more diversified and more drastic reaction conditions. Optimizations of derivatization conditions were carried out with the OHB dimer **1** as a model system, while compounds derived from HB 16mer **2** and 32mer **3** were the actual target molecules.

Water-Soluble OHB Derivatives. In *Scheme 1*, the synthesis of arginine- and glucose-coupled OHBs is outlined. For introducing arginine, we used the commercially available tri-Z-protected L-arginine derivative[6]), which was coupled with OHB esters **1**–**3**, using DCC/DMAP (dicyclohexylcarbodiimide/4-(dimethylamino)pyridine) in

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[3]) The water solubility of the cyclic triolide of HB was determined to be *ca.* 9 g/l [10].

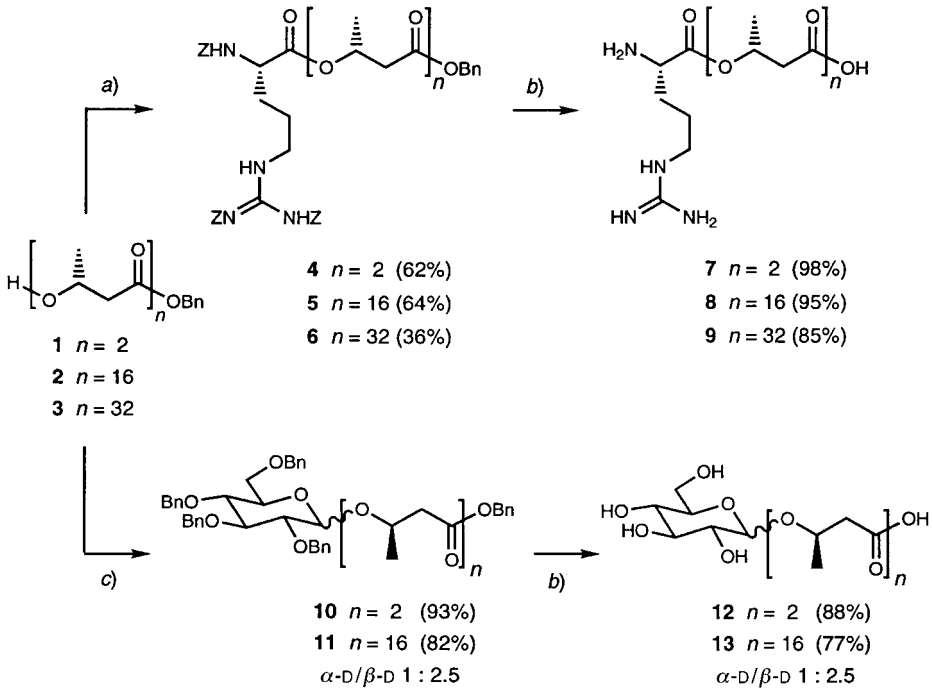
[4]) The formation of defined supramolecular aggregates, as, *e.g.*, micelles, has not been observed.

[5]) An analysis providing information about the amount of (*R*)- or (*S*)-3-hydroxybutanoate and -pentanoate in biological samples has been developed in our laboratory and will be described elsewhere.

[6]) Z = (Benzyloxy)carbonyl, Fmoc = (9*H*-fluoren-9-ylmethoxy)carbonyl, Pmc = (2,2,5,7,8-pentamethylchroman-6-yl)sulfonyl, Boc = (*tert*-butoxy)carbonyl.

CH_2Cl_2, to give the conjugates **4** (62%), **5** (64%), and **6** (36%). By using the *N*-Fmoc-N^{ω}-Pmc-protected arginine under otherwise similar conditions, the formation of several by-products was observed. Coupling with the *N*-Z-N^{ω}-NO_2-protected arginine derivative yielded the desired product in good yield (not shown), however, the subsequent deprotection did not work in our case. Hydrogenolytic debenzylation of compounds **4**–**6** with H_2 and Pd/C produced the fully deprotected compounds **7**–**9** in excellent yields.

Scheme 1. *Synthesis of Water-Solubilized HB Derivatives*



a) Tri-Z-L-arginine, DCC, DMAP, CH_2Cl_2, 0°. *b*) H_2, Pd/C, CF_3CH_2OH, r.t. *c*) 2,3,4,6-Tetra-*O*-benzyl-D-glucosyl trichloroacetimidate, $BF_3 \cdot Et_2O$, CH_2Cl_2, 0°.

DCC = dicyclohexylcarbodiimide, DMAP = 4-(dimethylamino)pyridine

Coupling of an OHB with a carbohydrate moiety was achieved by following the glycosylation procedure introduced by *Schmidt* [16]. Reaction of the hydroxy esters **1** and **2** with tetra-*O*-benzyl-D-glucosyl trichloroacetimidate, readily available in one step from tetra-*O*-benzyl-D-glucose, gave the fully benzyl-protected conjugates **10** (93%) and **11** (82%), respectively (*Scheme 1*). We were not able to separate the anomers by flash chromatography, but for the intended studies, the configuration has no significance. Hydrogenolytic cleavage of the benzyl ester and ethers in **10** and **11** with H_2 and Pd/C gave the desired products **12** (88%) and **13** (77%), respectively.

The water solubility of the OHB conjugates **8**, **9**, and **13** was compared with those of their precursors **2** and **3** (*Table*). Thus, the solubility of the 16mer was enhanced from

essentially zero (see **2**) to 1.43 g/l by arginine and to 0.94 g/l by glucose derivatization (see **8** and **13**, resp.) Arginine modification of the 32mer **3** resulted in a water solubility of 0.46 g/l (see **9**). These values point to a reasonably amphiphilic character of the OHB conjugates, Indeed, they are no longer soluble in pure CH_2Cl_2 as are their precursors, and CH_2Cl_2/MeOH mixtures are required to dissolve them.

Table. *Water Solubility of OHB Derivatives*

	n	Water solubility[a]) [g/l]
2	16	≤ 0.1
3	32	≤ 0.1
8	16	1.43 ($\pm 2\%$)
9	32	0.46 ($\pm 8\%$)
13	16	0.94 ($\pm 9\%$)

[a]) The values were determined as follows: A suspension of OHB material (in excess) in dist. H_2O was stirred for 1 d at r.t., then a part of the suspension was centrifugated, and an aliquot of the supernatant was taken. The solvent was carefully evaporated (10^{-9} bar), and the amount of solid residue was analyzed by weight.

Fluorescence-Labelled OHB Derivatives. Coupling of the hydroxy ester **1** with 7-(dimethylamino)coumarin-4-acetic acid (= 7-(dimethylamino)-2-oxo-2*H*-1-benzopyran-4-acetic acid) was tried under various reaction conditions[7]) (*Scheme 2*). The best result was obtained by applying the *Mukaiyama* method (*Entry 5*) [17] which yielded compound **14** in 30% yield.

The unexpectedly low yields of **14** obtained under various reaction conditions might be a consequence of the solvent mixtures employed (DMF/CH_2Cl_2; MeCN/CH_2Cl_2); esterification rates often tend to be influenced by solvent effects. Coupling of OHB **2** under similar conditions (*Scheme 2*, *Entry 5*) gave the fluorescence-labelled compound **15** (21%) (*Scheme 3*).

Hydrogenolysis of **15** was carried out in DMF with Pd/C as the catalyst and cyclohexa-1,4-diene as the hydrogen source [18] and gave acid **16** in 50% yield. Attempts to debenzylate with H_2 and Pd/C led to the destruction of the coumarin moiety. Coupling of OHB **1** and **2** with dansyl chloride (= 5-(dimethylamino)-naphthalene-1-sulfonyl chloride) under basic conditions[8]) gave the fluorescent compounds **17** (15%) and **18** (12%), attempted debenzylation of which, however, failed completely[9]).

Because of the problems encountered with some of the coupling reactions (see above), we decided to apply an alternative strategy: esterification of the OHBs **1**–**3** with a glycine spacer and subsequent functionalization of the spacer amino group[10])

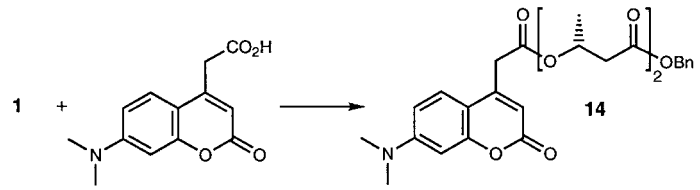
[7]) Attempts to prepare the acid chloride of 7-(dimethylamino)coumarin-4-acetic acid with oxalyl or thionyl chloride failed.

[8]) The use of various bases, solvents, and reaction temperatures did not lead to better results.

[9]) Hydrogenolysis with H_2 and Pd/C gave OHBs with free carboxy and hydroxy function, resulting from benzyl-ester and S–O bond cleavage. Catalytic transfer hydrogenolysis (Pd/C, cyclohexa-1,4-diene, DMF) gave OHBs with α,β-unsaturated carbonyl functionalities, resulting from a β-elimination of dansyl sulfate.

[10]) Because of the higher nucleophilicity of the amino group and the enhanced stability of the newly formed amide bond, the coupling reaction was expected to work better, what it actually did.

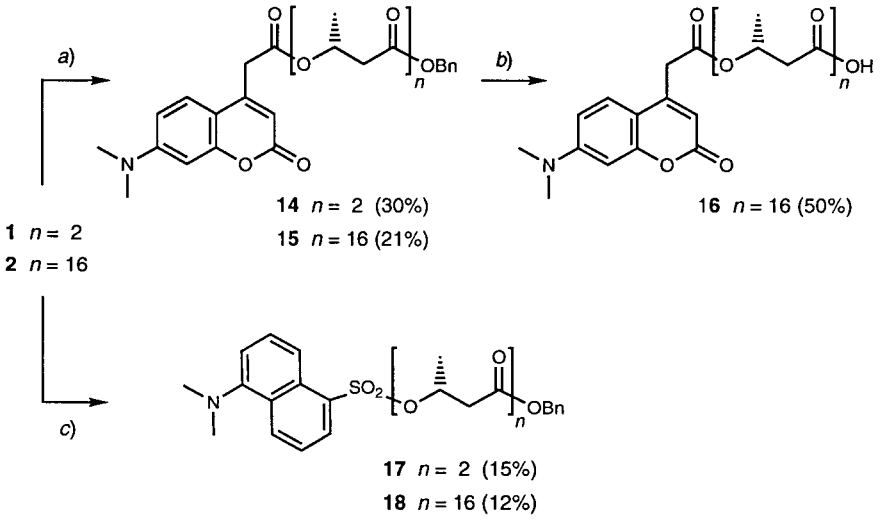
Scheme 2. *Coupling of the HB Dimer* **1** *with 7-(Dimethylamino)coumarin-4-acetic Acid*



Entry	Reaction conditions	Yield [%][a]) of **14**
1	DMAP (0.1 equiv.), DCC	24
2	DMAP, (0.5 equiv.), DCC	6
3	di(2-pyridyl) disulfide, PPh_3, $CuBr_2$	6
4	BOP-Cl[b]), Et_3N	6
5	2-chloro-1-methylpyridinium iodide, Et_3N	30
6	2-chloro-1-methylpyridinium iodide, DMAP (cat.)	–
7	PPh_3, CCl_4, pyridine	2

[a]) Based on material isolated from SiO_2 chromatography. [b]) BOP-Cl = bis(2-oxo-oxazolidin-3-yl)phosphinic chloride.

Scheme 3. *Synthesis of Fluorescent OHB Derivatives*

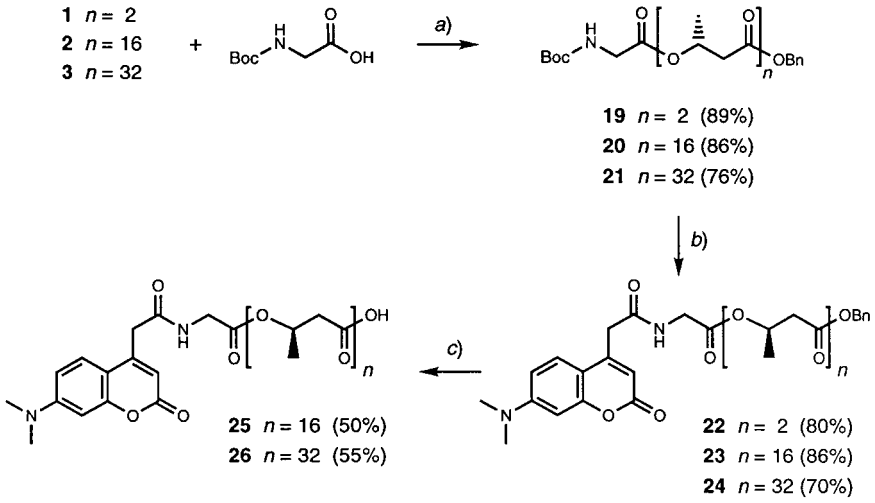


a) 7-(Dimethylamino)coumarin-4-acetic acid, 2-chloro-1-methylpyridinium iodide, Et_3N, CH_2Cl_2, MeCN, r.t. *b*) Cyclohexa-1,4-diene, Pd/C, DMF, r.t. *c*) 5-(Dimethylamino)naphthalene-1-sulfonyl chloride, DMAP, 50°, $ClCH_2CH_2Cl$.

with the fluorescent building block (*Scheme 4*). For the introduction of the glycine moiety, we coupled commercial *N*-Boc-glycine[6]) with OHBs **1**–**3** using DCC/DMAP in CH_2Cl_2, to give the conjugates **19**–**21** in excellent yields of 89, 86, and 76%, respectively. Removal of the Boc group (CF_3COOH/CH_2Cl_2) and subsequent standard peptide coupling of the trifluoroacetates derived from **19**–**21**, using EDC/HOBt (*N*-[3-

(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride/1-hydroxy-1*H*-benzotriazole) [19] in $CHCl_3$/DMF, with 7-(dimethylamino)coumarin-4-acetic acid gave conjugates **22** (80%), **23** (96%), and **24** (70%), respectively. Catalytic transfer hydrogenolysis (Pd/C, cyclohexa-1,4-diene, DMF) of **23** yielded **25** (50%), whereas cleavage of the benzyl ester of **24** was performed with H_2 and Pd/$BaSO_4$ to afford the free acid **26** in 55% yield.

Scheme 4. *Synthesis of HB Derivatives Linked to a Coumarin* via *a Glycine Spacer*



a) DCC, DMAP, CH_2Cl_2, 0°. *b*) 1. CF_3COOH, CH_2Cl_2, 0°, 2. Et_3N, $CHCl_3$, 0°; 3. 7-(dimethylamino)coumarin-4-acetic acid, EDC, HOBt, DMF, $CHCl_3$, 0° → r.t. *c*) Cyclohexa-1,4-diene, Pd/C, DMF, r.t. or H_2, Pd/$BaSO_4$, CF_3CH_2OH, r.t. EDC = *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride

Analysis of the UV/VIS spectra of the coumarin-labelled compounds **25** and **26** revealed a coumarin-specific absorption with a maximum at 376 nm and a molar extinction coefficient ε of 15837 M^{-1} cm^{-1} (± 2.5%) in CF_3CH_2OH[11]). The high molar extinction coefficient and the coumarin absorption in a region, where most lipids do not absorb, make these compounds well-suited for detection by UV/VIS or fluorescence spectrometry in minute amounts in lipid membranes.

Biotin-Linked OHBs. For the reaction of an OHB hydroxy group with the acid functionality of (+)-biotin (=(3a*S*,4*S*,6a*R*)-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanoic acid), we intended to activate the acid by conversion to an acid chloride, a process which succeeded neither with oxalyl chloride nor with thionyl chloride. On the other hand, the (+)-biotin ester with *N*-hydroxysuccinimide [20] did not react with OHB **1** under various conditions tested. We managed to couple (+)-biotin with the OHBs **1** and **2**, using DCC/DMAP in CH_2Cl_2/DMF, to give the derivatives **27** and **28** in poor yields (*Scheme 5*). Hydrogenolytic cleavage over

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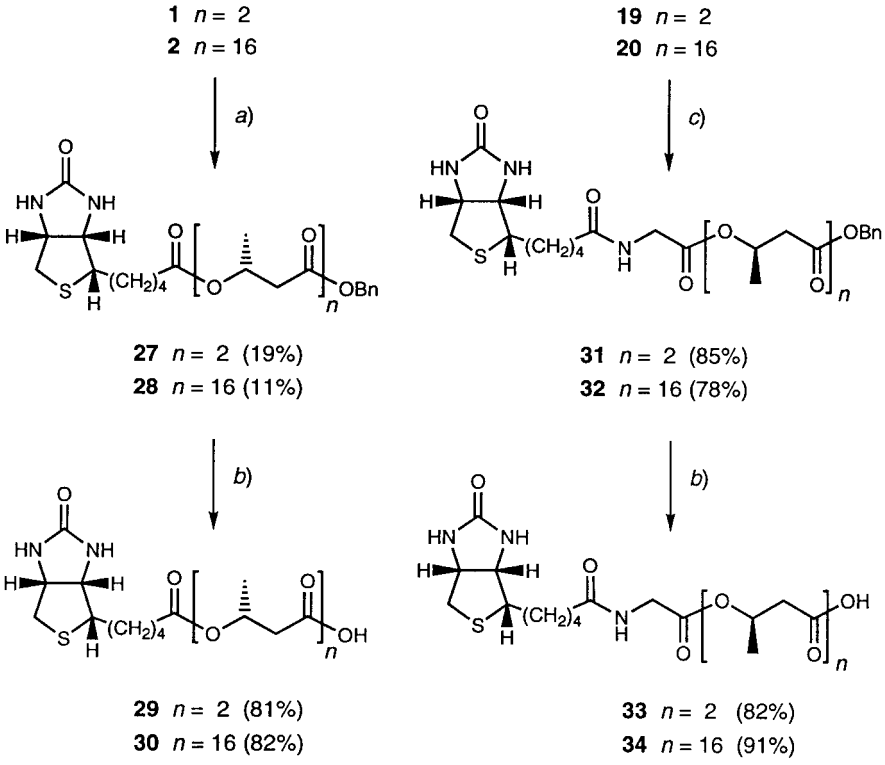
11) For comparison: the 32mer HB with free carboxy and hydroxy function has a UV absorption maximum at 214 nm and a molar extinction coefficient ε of 3326 M^{-1} cm^{-1} (± 2.5%) in CF_3CH_2OH. The 376-nm coumarin absorption does not give rise to a *Cotton* effect of compound **26**.

Pd/C[12]) of the benzyl esters in **27** and **28** gave the 'biotinylated' acids **29** and **30**, both in *ca.* 82% yield. In view of the low coupling yields, we decided to use the strategy applied for the introduction of coumarin: reaction of the glycinated OHB derivatives **19** and **20** with (+)-biotin, using EDC/HOBt in $CHCl_3$/DMF, gave the 'biotinylated' compounds **31** (85%) and **32** (78%) (*Scheme 5*). Hydrogenolytic debenzylation of compounds **31** and **32** with H_2 and Pd/C yielded the acids **33** (82%) and **34** (91%), respectively.

Scheme 5. *Synthesis of 'Biotinylated' HB Derivatives*



a) (+)-Biotin, DCC, DMAP, DMF, CH_2Cl_2, 0°. *b*) H_2, Pd/C, CF_3CH_2OH, r.t. *c*) 1. CF_3COOH, CH_2Cl_2, 0°; 2. Et_3N, $CHCl_3$, 0°; 3. (+)-biotin, EDC, HOBt, DMF, $CHCl_3$, 0° → r.t.

In conclusion, the experiments described herein provide, for the first time, pure (*R*)-3-hydroxybutanoate oligomers (OHBs) derivatized in such a way that they become somewhat H_2O-soluble, at least to the extent required for biochemical experiments (0.5–1.5 g/l). Furthermore, we have synthesized OHB derivatives which can be detected in tiny amounts by direct (the 'coumarinylated' ones) or by indirect (the 'biotinylated' ones) fluorescence measurements. This will enable us – and hopefully also others – to investigate in more detail the intriguing and widely unknown

[12]) It is known that sulfur-containing compounds may poison the Pd/C catalyst, but in our work, this was not the case [21].

role which the simple biomacromolecules consisting of less than 200 3-hydroxybutanoate residues (cPHB) play in biology.

We thank *Zeneca Bio Products*, Billingham, GB, for supplying P(3-HB) and the *Fonds der Basler Chemischen Industrie* for a scholarship granted to *M.G.F.*

Experimental Part

1. *Abbreviations.* BOP-Cl (bis(2-oxooxazolidin-3-yl)phosphinic chloride), DCC (dicyclohexylcarbodiimide), DMAP (4-(dimethylamino)pyridine), EDC (*N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride), HOBt (1-hydroxy-1*H*-benzotriazole), h.v. (high vacuum, $10^{-4}-10^{-6}$ mbar).

2. *General.* All solvents were either *puriss p.a.* quality or distilled over appropriate drying reagents. CH_2Cl_2 and DMF were stored over 4-Å molecular sieves. All other reagents were used as purchased from *Fluka* or *Senn*. Compounds **1**–**3** were synthesized as described in [15]. For h.v. $< 10^{-4}$ mbar, a turbomolecular pump *Balzers TSH065* was employed. TLC: *Merck* silica gel *60* F_{254} anal. plates; detection either with UV or by dipping into a soln. of I_2 (30 g), KI (2 g), EtOH (200 ml), H_2O (200 ml) and drying in the air. Flash chromatography (FC): *Merck* silica gel *60* (40–63 μm). M.p.: *Büchi 510*; uncorrected. Optical rotations: 10-cm, 1-ml cell, at r.t.; *Perkin-Elmer-241* polarimeter. UV/VIS Spectra: *Varian-Cary-1E-UV-VIS* spectrophotometer; λ_{max} in nm (ε). IR Spectra: *Perkin-Elmer-1600-FT* spectrophotometer. ^{1}H-NMR: *Bruker-AMX-II-500* (500 MHz), *AMX-400* (400 MHz), *ARX-300* (300 MHz), or *Varian-Gem-200* (200 MHz) spectrometer. ^{13}C-NMR: *Bruker-AMX-500* (125 MHz), *AMX-400* (100 MHz), *ARX-300* (75 MHz), or *Varian-Gem-200* (50 MHz) spectrometer; in $CDCl_3$, unless specified otherwise. MS: *VG TRIBRID* for EI; *VG ZAB2-SEQ* for liquid secondary ionization (fast-atom bombardment) (LSI (FAB)) with 3-nitrobenzyl alcohol as matrix; *Bruker-Reflex-MALDI TOF* spectrometer for matrix-assisted laser desorption time-of-flight (MALDI-TOF). Elemental analyses were conducted by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

3. *General Procedure for the Coupling Using EDC* (*GP I*). According to [19], a stirred soln. of the trifluoroacetate salt (1 equiv.) in $CHCl_3$ at 0° (ice-bath) under Ar was treated with Et_3N (5 equiv.). This mixture was added to a stirred soln. of the acid (1 equiv.) in DMF at 0° (ice-bath) under Ar. HOBt (1.2 equiv.) and EDC (1.2 equiv.) were added successively. The mixture was allowed to warm to r.t., and stirring was continued for 16 h. The mixture was evaporated, the residue dissolved in CH_2Cl_2, the soln. washed with 1N HCl, aq. sat. $NaHCO_3$, and sat. NaCl soln., dried ($MgSO_4$), and evaporated, and the residue purified by FC.

4. *General Procedure for the Coupling Using DCC* (*GP II*). According to [22], a 0°-cold soln. of DCC (1.1 equiv.) and DMAP (0.1 equiv.) in CH_2Cl_2 was added under Ar to a stirred soln. of the alcohol (1 equiv.) and the acid (1 equiv.) in CH_2Cl_2 at −10° (ice/salt-bath). The mixture was allowed to warm to r.t., and stirring was continued for 24 h. The mixture was filtered through *Celite* and washed with 1N HCl, aq. sat. $NaHCO_3$, and NaCl soln., the org. phase dried ($MgSO_4$) and evaporated, and the residue purified by FC.

5. *General Procedure for the Cleavage of Benzyl Ester and Benzyl Ether* (*GP III*). *GP IIIa:* According to [23], the benzyl-ester or -ether-protected oligomer was dissolved in CF_3CH_2OH at r.t., then 10% Pd/C was added, and hydrogenation was carried out under H_2 and vigorous stirring. The reaction went to completion within 5 to 20 h. The mixture was filtered through *Celite* and evaporated. The residue was purified by FC.

GP IIIb: According to [23], the benzyl-ester or -ether-protected oligomer was dissolved in CF_3CH_2OH at r.t., then 10% Pd/$BaSO_4$ was added, and hydrogenation was carried out under H_2 and vigorous stirring for 8 h. The mixture was filtered through *Celite* and evaporated. The residue was purified by FC.

GP IIIc: According to [18], a soln. of the benzyl-ester-protected oligomer and cyclohexa-1,4-diene (20 equiv.) in DMF was treated with 10% Pd/C under Ar. After 12–24 h stirring, the mixture was filtered through *Celite* and evaporated. The residue was purified by FC.

6. *General Procedure for the Boc-Deprotection of Amino Acids* (*GP IV*). According to [24], a stirred soln. of the amino-acid derivative in CH_2Cl_2 was treated at 0° (ice-bath) under Ar with an equal volume of CF_3CO_2H. The mixture was allowed to warm to r.t., and stirring was continued for 1.5 h. The mixture was evaporated and the residue dried under h.v. The salts with CF_3CO_2H were used without further purification or characterization.

7. *Arginine-HB Conjugates* **4**–**9**. *Benzyl (3*R*)-3-{[(3'*R*)-3'-{[(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-5-{2,3-bis[(benzyloxy)carbonyl]guanidino}pentanoyl]oxy}butanoyl]oxy}butanoate* (**4**). Compound **1** (236 mg, 0.85 mmol) was transformed according to *GP II* with commercially available Z-Arg(Z_2)-OH (490 mg, 0.85 mmol) in CH_2Cl_2 (20 ml). FC (CH_2Cl_2/Et_2O 11:1) yielded **4** (440 mg, 62%). Colorless microcrystalline solid. M.p. 87–89°. R_f 0.6 (CH_2Cl_2/Et_2O 11:1). $[\alpha]_D = +2.50$ ($c = 0.6$, $CHCl_3$). IR ($CHCl_3$): 3396*w*, 3008*w*,

2976*w*, 1722*s*, 1611*w*, 1511*w*, 1455*w*, 1381*w*, 1101*w*, 1048*w*, 930*w*. ^{1}H-NMR (400 MHz): 9.45 (br., NH); 9.26 (br., NH); 7.41–7.29 (*m*, 20 arom. H); 5.63 (*d*, *J* = 8.26, NH); 5.31–5.26 (*m*, 2 CH); 5.12 (*s*, PhCH_2O); 5.11 (*s*, PhCH_2O); 5.08 (*s*, PhCH_2O); 5.07 (*s*, PhCH_2O); 4.40–4.25 (*m*, CH); 4.01–3.97 (*m*, CH_2); 2.67, 2.52 (*AB* of *ABX*, J_{AB} = 15.81, J_{AX} = 7.95, J_{BX} = 5.15); 2.51, 2.31 (*AB* of *ABX*, J_{AB} = 15.72, J_{AX} = 8.99, J_{BX} = 5.71); 1.84–1.71 (*m*, CH_2); 1.70–1.62 (*m*, CH_2); 1.27 (*d*, *J* = 6.33, Me); 1.18 (*d*, *J* = 6.26, Me). ^{13}C-NMR (100 MHz): 171.23; 170.01; 169.07; 163.85; 160.50; 155.81; 136.91; 135.64; 135.65; 128.80; 128.55; 128.47; 128.37; 128.34; 128.31; 128.09; 128.04; 127.93; 127.79; 68.91; 68.51; 67.67; 66.96; 66.82; 66.51; 53.74; 44.19; 40.61; 40.51; 29.20; 24.58; 19.75; 19.50. LSI-MS: 841.3 (1, $[M+2H]^+$), 840.3 (52, $[M+H]^+$), 839.3 (100, M^+), 705.3(15), 687.3(24), 154.0(27), 137.0(19), 136.0(25), 107.0(16). Anal. calc. for $C_{45}H_{50}N_4O_{12}$: C 64.43, H 6.01, N 6.68; found: C 64.26, H 5.86, N 6.79.

*α-[(2*S*)-2-[[(Benzyloxy)carbonyl]amino]-5-{2,3-bis[(benzyloxy)carbonyl]guanidino}pentanoyl]-ω-(benzyloxy)hexadecakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**5**). Compound **2** (1.49 g, 1.00 mmol) was transformed according to *GP II* with commercially available Z-L-Arg(Z_2)-OH (0.58 g, 1.00 mmol) in CH_2Cl_2 (50 ml). FC (CH_2Cl_2/Et_2O 3:1) yielded **5** (1.3 g, 64%). White solid. M.p. 105–106°. R_f 0.5 (CH_2Cl_2/Et_2O 3:1). $[\alpha]_D$ = +1.62 (*c* = 0.9, $CHCl_3$). IR ($CHCl_3$): 3387*w*, 3033*w*, 2985*w*, 1738*s*, 1611*w*, 1512*w*, 1457*w*, 1382*w*, 1306*m*, 1257*m*, 1178*m*, 1101*m*, 1059*m*, 979*w*. ^{1}H-NMR (400 MHz): 9.43 (br., NH); 9.25 (br., NH); 7.38–7.29 (*m*, 20 arom. H); 5.63 (*d*, *J* = 8.39, NH); 5.32–5.20 (*m*, 16 CH); 5.12 (*s*, 2 PhCH_2O); 5.10 (*s*, PhCH_2O); 5.06 (*s*, PhCH_2O); 4.33–4.31 (*m*, CH); 3.99–3.97 (*m*, CH_2); 2.71–2.37 (*m*, 16 CH_2); 1.81–1.70 (*m*, CH_2); 1.70–1.64 (*m*, CH_2); 1.28–1.18 (*m*, 16 CH_3). ^{13}C-NMR (100 MHz): 171.20; 169.87; 169.21; 169.11; 169.02; 160.48; 155.80; 136.91; 136.39; 135.67; 128.78; 128.56; 128.46; 128.37; 128.31; 128.09; 128.05; 127.92; 127.77; 68.90; 68.50; 67.66; 67.57; 66.93; 66.54; 40.76; 40.66; 40.63; 40.60; 24.55; 19.78; 19.73; 19.70; 19.66; 19.53. LSI-MS: 2066.8 (1, $[M+Na]^+$), 2065.6 (3, $[M+Na-H]^+$), 2044.9 (100, $[M+H]^+$), 1910.6(37), 1892.7(11), 155.1(17), 154.1(12), 137.0(11), 136.0(11). Anal. calc. for $C_{101}H_{134}N_4O_{40}$: C 59.34, H 6.61, N 2.74; found: C 59.49, H 6.51, N 2.80.

*α-[(2*S*)-2-[[(Benzyloxy)carbonyl]amino]-5-{2,3-bis[(benzyloxy)carbonyl]guanidino}pentanoyl]-ω-(benzyloxy)dotriacontakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**6**). Compound **3** (872 mg, 0.31 mmol) was transformed according to *GP II* with commercially available Z-L-Arg(Z_2)-OH (192 mg, 0.37 mmol) in CH_2Cl_2 (50 ml). FC (CH_2Cl_2/Et_2O 2:1) yielded **6** (369 mg, 36%). White solid. M.p. 118–120°. R_f 0.7 (CH_2Cl_2/Et_2O 2:1). $[\alpha]_D$ = +1.32 (*c* = 0.8, $CHCl_3$). IR ($CHCl_3$): 2988*w*, 2936*w*, 1738*s*, 1506*w*, 1457*w*, 1382*w*, 1306*m*, 1261*m*, 1178*m*, 1133*w*, 1100*m*, 1059*m*, 980*w*. ^{1}H-NMR (500 MHz): 9.43 (br., NH); 9.24 (br., NH); 7.38–7.28 (*m*, 20 arom. H); 5.63 (*d*, *J* = 8.31, NH); 5.32–5.21 (*m*, 32 CH); 5.12 (*s*, 2 PhCH_2O); 5.10 (*s*, PhCH_2O); 5.06 (*s*, PhCH_2O); 4.34–4.32 (*m*, CH); 3.98–3.97 (*m*, CH_2); 2.71–2.38 (*m*, 32 CH_2); 1.81–1.72 (*m*, CH_2); 1.68–1.64 (*m*, CH_2); 1.29–1.18 (*m*, 32 Me). ^{13}C-NMR (125 MHz): 171.25; 169.91; 169.25; 169.15; 169.07; 163.87; 160.52; 155.85; 136.95; 136.44; 135.72; 134.70; 128.83; 128.61; 128.50; 128.41; 128.35; 128.13; 128.09; 127.96; 127.82; 68.94; 68.54; 67.78; 67.70; 67.63; 67.46; 67.24; 66.98; 66.84; 66.49; 53.79; 44.22; 40.81; 40.71; 40.68; 40.64; 33.97; 29.26; 25.63; 24.95; 24.60; 19.78; 19.74; 19.70; 19.57. MALDI-MS: 3445.7 ($[M+Na]^+$). Anal. calc. for $C_{165}H_{230}N_4O_{72}$: C 57.92, H 6.78, N 1.64; found: C 57.63, H 7.03, N 1.58.

*(3*R*)-3-{[(3′*R*)-3′-{[(2*S*)-2-Amino-5-guanidinopentanoyl]oxy}butanoyl]oxy}butanoic Acid* (**7**). Compound **4** (83 mg, 99 μmol) was hydrogenated according to *GP IIIa* in CF_3CH_2OH (30 ml): **7** (34 mg, 98%). Colorless, glassy solid. M.p. 84–86°. IR (NaCl film): 3675*w*, 3377*w*, 3029*w*, 2960*w*, 1737*s*, 1602*m*, 1519*m*, 1453*m*, 1384*m*, 1260*m*, 1102*w*, 1055*w*, 1012*w*. ^{1}H-NMR (400 MHz, $CDCl_3$/CD_3OD): 7.35–7.31 (*m*, NH); 5.30–5.13 (*m*, 2 CH); 4.17–4.06 (*m*, CH); 3.62–3.50 (*m*, CH_2); 2.70–2.23 (*m*, 2 CH_2); 1.95–1.87 (*m*, CH_2); 1.87–1.73 (*m*, CH_2); 1.28 (*d*, *J* = 6.10, Me); 1.21 (*d*, *J* = 6.19, Me). ^{13}C-NMR (100 MHz, $CDCl_3$/CD_3OD): 180.25; 178.35; 172.79; 158.42; 129.09; 70.65; 66.13; 66.29; 55.19; 46.40; 45.14; 44.99; 41.61; 29.26; 25.36; 23.11; 22.87; 20.29. ESI-MS: 347.3 (14, $[M+H]^+$), 298.3(100).

*α-[(2*S*)-2-Amino-5-guanidinopentanoyl]-ω-hydroxyhexadecakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**8**). Compound **5** (400 mg, 196 μmol) was hydrogenated according to *GP IIIa* in CF_3CH_2OH (20 ml): **8** (289 mg, 98%). White solid. M.p. 151–153°. IR (KBr): 3677*w*, 3379*w*, 3029*w*, 2959*w*, 1737*s*, 1601*w*, 1519*w*, 1454*w*, 1384*m*, 1260*m*, 1104*w*, 1056*w*, 1012*w*. ^{1}H-NMR (400 MHz, $CDCl_3$/CD_3OD): 5.41–5.36 (*m*, NH); 5.30–5.22 (*m*, 16 CH); 3.94–3.87 (*m*, CH); 3.29–3.25 (*m*, CH_2); 2.72–2.46 (*m*, 16 CH_2); 2.04–1.97 (*m*, CH_2); 1.81–1.76 (*m*, CH_2); 1.36 (*d*, *J* = 6.35, Me); 1.30 (*d*, *J* = 6.47, Me); 1.28 (*d*, *J* = 7.99, 4 Me); 1.28 (*d*, *J* = 6.31, 10 Me). ^{13}C-NMR (100 MHz, $CDCl_3$/CD_3OD): 172.79; 169.76; 169.58; 169.44; 169.41; 169.40; 169.33; 69.82; 67.94; 67.84; 67.79; 67.71; 67.61; 40.74; 40.64; 40.51; 40.40; 40.31; 27.47; 24.40; 19.66; 19.59. LSI-MS: 1580.2 (20); 1553.2 (68, $[M+2H]^+$), 1552.4 (100, $[M+H]^+$); 1551.7 (57, M^+). Anal. calc. for $C_{70}H_{110}NO_{34}\cdot CF_3CH_2OH$: C 50.69, H 6.63, N 3.20; found: C 50.31, H 6.75, N 3.49.

*α-[(2*S*)-2-Amino-5-guanidinopentanoyl]-ω-hydroxydotriacontakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**9**). Compound **6** (200 mg, 58 μmol) was hydrogenated according to *GP IIIa* in CF_3CH_2OH (10 ml): **9**

(145 mg, 85%). M.p. 141–142°. $[\alpha]_D = +0.92$ ($c = 0.8$, $CHCl_3$). IR ($CHCl_3$): 2988*w*, 2936*w*, 1737*s*, 1459*w*, 1383*m*, 1306*m*, 1134*w*, 1101*w*, 1059*w*, 980*w*. ^{1}H-NMR (500 MHz): 5.41–5.38 (*m*, NH); 5.30–5.22 (*m*, 32 CH); 3.48–3.42 (*m*, CH); 3.18–3.14 (*m*, CH_2); 2.63–2.45 (*m*, 32 CH_2); 1.98–1.81 (*m*, CH_2); 1.74–1.63 (*m*, CH_2); 1.31–1.21 (*m*, 32 Me). ^{13}C-NMR (125 MHz): 176.50; 174.69; 169.71; 169.41; 169.34; 169.30; 169.27; 169.18; 160.27; 158.02; 128.36; 69.97; 68.03; 67.96; 67.87; 67.64; 67.25; 53.93; 49.20; 44.11; 41.08; 41.02; 40.80; 40.50; 33.95; 30.02; 25.63; 25.42; 24.95; 20.09; 19.78. MALDI-MS: 2953.9 ($[M+Na]^+$), 2930.1 ($[M+H]^+$).

8. *Glycosylated HB Oligomers* **10**–**13**. *Benzyl (3*R*)-3-{[(3′*R*)-3′-[(2,3,4,6-Tetra-*O*-benzyl-α/β-*D*-glucopyranosyl)oxy]butanoyl}oxy}butanoate* (**10**). A soln. of 2,3,4,6-tetra-*O*-benzyl-α-D-glucosyl trichloroacetimidate (520 mg, 0.76 mmol), prepared according to [16], and **1** (420 mg, 1.52 mmol) in CH_2Cl_2 (50 ml) at −20° under Ar was treated slowly with $BF_3 \cdot Et_2O$ (0.17 ml, 0.76 mmol). After 2.5 h stirring, the mixture was washed with aq. sat. $NaHCO_3$ and NaCl soln. The org. phase was dried ($MgSO_4$) and evaporated. FC (Et_2O/pentane 4:3) yielded **10** (567 mg, 93%), β-D/α-D 2.5:1. Colorless oil. R_f 0.6 (Et_2O/pentane 4:3). $[\alpha]_D = +3.67$ ($c = 1.4$, $CHCl_3$). IR ($CHCl_3$): 3684*w*, 3621*m*, 3468*w*, 3008*s*, 2976*s*, 1734*m*, 1519*m*, 1477*w*, 1422*m*, 1391*w*, 1047*m*, 929*m*. ^{1}H-NMR (400 MHz): 7.36–7.22 (*m*, 25 arom. H); 5.32–5.24 (*m*, CH); 5.09 (*s*, PhCH_2O); 4.97 (*d*, $J = 3.73$, H–C(1)(α)); 4.95 (*d*, $J = 10.91$, 1 H, PhCH_2O); 4.91 (*d*, $J = 10.93$, 1 H, PhCH_2O); 4.82 (*d*, $J = 10.71$, 1 H, PhCH_2O); 4.80 (*d*, $J = 10.54$, 1 H, PhCH_2O); 4.77 (*d*, $J = 10.84$, 2 H, PhCH_2O); 4.68 (*d*, $J = 10.98$, 2 H, PhCH_2O); 4.62 (*d*, $J = 12.79$, 1 H, PhCH_2); 4.60 (*d*, $J = 12.15$, 1 H, PhCH_2); 4.55 (*d*, $J = 11.39$, 1 H, PhCH_2); 4.52 (*d*, $J = 10.83$, 1 H, PhCH_2O); 4.52 (*d*, $J = 12.27$, 1 H, PhCH_2O); 4.46 (*d*, $J = 7.75$, H–C(1)(β)); 4.46 (*d*, $J = 10.24$, 1 H, PhCH_2O); 4.46 (*d*, $J = 12.62$, 1 H, PhCH_2); 4.29–4.21 (*m*, H–C(β)); 4.16–4.11 (*m*, H–C(α)); 3.94 (*dd* (*t*), $J = 9.32$, H–C(3)(α)); 3.86–3.81 (*m*, H–C(5)(α)); 3.73 (*dd*, $J = 3.69$, 10.48, H–C(6)(β)); 3.70–3.63 (*m*, H–C(3)(β), H–C(4)(α), H–C(6)(β), 2 H–C(6)(α)); 3.60 (*dd*, $J = 8.67$, 11.33, H–C(4)(β)); 3.52 (*dd*, $J = 3.69$, 9.71, H–C(2)(α)); 3.43–3.40 (*m*, H–C(5)(β)); 3.39 (*dd* (*t*), $J = 8.38$, H–C(2)(β)); 2.81–2.39 (*m*, 2 CH_2); 1.28 (*d*, $J = 6.29$, Me); 1.25 (*d*, $J = 6.17$, Me). ^{13}C-NMR (100 MHz): 170.10; 170.00; 169.94; 138.65; 138.46; 138.23; 138.18; 128.57; 128.55; 128.40; 128.34; 128.14; 128.05; 127.93; 127.87; 127.84; 127.75; 127.69; 127.65; 127.54; 127.50; 101.78; 84.73; 82.17; 81.92; 79.84; 76.68; 75.66; 75.60; 74.96; 74.84; 74.81; 73.44; 73.40; 71.87; 68.89; 67.40; 66.41; 42.85; 41.73; 40.69; 40.62; 21.37; 19.80. LSI-MS: 826.3 (13, $[M+Na+H]^+$), 825.3 (25, $[M+Na]^+$), 803.5 (15, $[M+H]^+$), 802.3 (37, M^+), 801.2 (72, $[M-H]^+$), 182.1 (19), 181.1 (100), 107.0 (17), 105.0 (17). Anal. calc. for $C_{49}H_{54}O_{10}$: C 73.30, H 6.78; found: C 73.26, H 6.87.

*α-(2,3,4,6-Tetra-*O*-benzyl-α/β-*D*-glucopyranosyl)-ω-(benzyloxy)hexadecakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**11**). A soln. of 2,3,4,6-tetra-*O*-benzyl-α-D-glucosyl trichloroacetimidate (0.44 g, 0.64 mmol) and **2** (1.43 g, 0.97 mmol) in CH_2Cl_2 (80 ml) at −20° under Ar was treated slowly with $BF_3 \cdot Et_2O$ (0.14 ml, 0.65 mmol). After 2.5 h stirring, the mixture was washed with aq. sat. $NaHCO_3$ and NaCl soln. The org. phase was dried ($MgSO_4$) and evaporated. FC (CH_2Cl_2/Et_2O 6:1) yielded **11** (1.06 g, 82%), β-D/α-D 2.5:1. White solid. M.p. 89–90°. R_f 0.3 (CH_2Cl_2/Et_2O 6:1). $[\alpha]_D = +2.01$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3520*w*, 2985*w*, 2937*w*, 1737*s*, 1456*w*, 1382*m*, 1306*m*, 1264*m*, 1178*m*, 1100*m*, 1060*m*, 979*w*. ^{1}H-NMR (400 MHz): 7.36–7.25 (*m*, 25 arom. H); 5.31–5.22 (*m*, 15 CH); 5.12 (*s*, PhCH_2O); 4.97 (*d*, $J = 3.28$, H–C(1)(α)); 4.96 (*d*, $J = 11.71$, 1 H, PhCH_2O); 4.92 (*d*, $J = 10.92$, 1 H, PhCH_2O); 4.90 (*d*, $J = 10.97$, 2 H, PhCH_2O); 4.82 (*d*, $J = 10.71$, 1 H, PhCH_2O); 4.80 (*d*, $J = 10.83$, 1 H, PhCH_2O); 4.77 (*d*, $J = 10.97$, 2 H, PhCH_2O); 4.70 (*d*, $J = 12.08$, 1 H, PhCH_2O); 4.68 (*d*, $J = 10.94$, 1 H, PhCH_2O); 4.61 (*d*, $J = 12.20$, 1 H, PhCH_2O); 4.61 (*d*, $J = 12.24$, 1 H, PhCH_2O); 4.53 (*d*, $J = 10.85$, 1 H, PhCH_2O); 4.53 (*d*, $J = 12.09$, 1 H, PhCH_2O); 4.48 (*d*, $J = 7.81$, H–C(1)(β)); 4.46 (*d*, $J = 12.81$, 1 H, PhCH_2O); 4.30–4.26 (*m*, H–C(β)); 4.17–4.13 (*m*, H–C(α)); 3.94 (*dd* (*t*), $J = 9.36$, H–C(3)(α)); 3.90–3.82 (*m*, H–C(5)(α)); 3.74 (*dd*, $J = 2.88$, 9.68, H–C(6)(β)); 3.72 (*dd*, $J = 2.13$, 10.76, H–C(6)(α)); 3.69 (*dd*, $J = 5.81$, 10.11, H–C(6)(β)); 3.67–3.57 (*m*, H–C(3)(β), H–C(4)(α), H–C(4)(β), H–C(6)(α)); 3.53 (*dd*, $J = 3.65$, 9.70, H–C(2)(α)); 3.44–3.41 (*m*, H–C(5)(β)); 3.40 (*dd* (*t*), $J = 8.41$, H–C(2)(β)); 2.71–2.38 (*m*, 16 CH_2); 1.29–1.24 (*m*, 9 Me); 1.27 (*d*, $J = 6.24$, 6 Me); 1.24 (*d*, $J = 6.31$, Me). ^{13}C-NMR (100 MHz); 170.07; 169.91; 169.15; 128.60; 128.45; 128.37; 128.36; 128.17; 128.08; 127.96; 127.90; 127.73; 127.72; 127.66; 127.59; 101.83; 84.76; 82.18; 74.98; 74.86; 74.82; 73.42; 71.90; 67.70; 67.62; 67.57; 67.37; 66.49; 42.86; 40.87; 40.81; 40.68; 19.82; 19.78; 19.74. LSI-MS: 2140.8 (26, $[M+Cs]^+$), 2046.6 (4, $[M+K]^+$), 2030.5 (69, $[M+Na]^+$), 2007.0 (14, M^+), 1576.1 (30), 1485.7 (39), 182.1 (21), 181.1 (85), 173.1 (21), 156.1 (20), 155.0 (100), 154.0 (56), 138.0 (23), 137.0 (46), 136.0 (48). Anal. calc. for $C_{105}H_{138}O_{38}$: C 62.80, H 6.93; found: C 62.81, H 6.92.

*(3*R*)-3-{[(3′*R*)-3′-[(α/β-*D*-Glucopyranosyl)oxy]butanoyl}oxy}butanoic Acid* (**12**). Compound **10** (160 mg, 0.20 mmol) was hydrogenated according to *GP IIIa* in CF_3CH_2OH (40 ml). FC (CH_2Cl_2/MeOH/H_2O 6:2.5:0.25) yielded **12** (62 mg, 88%), β-D/α-D 2.5:1. Colorless oil. R_f 0.2 (CH_2Cl_2/MeOH/H_2O 6:2.5:0.25). IR (KBr): 3503*w*, 2979*w*, 2933*w*, 1738*s*, 1448*w*, 1383*m*, 1305*m*, 1178*m*, 1136*m*, 1100*m*, 1067*s*, 977*w*. ^{1}H-NMR (400 MHz, CD_3OD): 5.28–5.22 (*m*, CH); 4.94 (*d*, $J = 3.85$, H–C(1)(α)); 4.34 (*d*, $J = 7.75$, H–C(1)(β)); 4.29–

4.23 (*m*, H–C(*β*)); 4.20–4.13 (*m*, H–C(*α*)); 3.84 (*dd*, *J* = 2.00, 11.88, H–C(6)(*β*)); 3.79 (*dd*, *J* = 2.09, 13.66, H–C(6)(*α*)); 3.76–3.62 (*m*, H–C(3)(*α*), H–C(5)(*α*), H–C(6)(*α*), H–C(6)(*β*)); 3.58 (*dd* (‘*t*’), *J* = 9.34, H–C(3)(*β*)); 3.48–3.24 (*m*, H–C(4)(*α*), H–C(4)(*β*), H–C(5)(*β*)); 3.11 (*dd* (‘*t*’), *J* = 8.42, H–C(2)(*β*)); 2.70, 2.50 (*AB* of *ABX*, J_{AB} = 15.88, J_{AX} = 6.58, J_{BX} = 5.76); 2.60, 2.44 (*AB* of *ABX*, J_{AB} = 15.40, J_{AX} = 7.89, J_{BX} = 6.68); 1.29 (*d*, *J* = 6.29, Me); 1.24 (*d*, *J* = 6.21, Me). ^{13}C-NMR (100 MHz, CD_3OD): 172.65; 172.63; 102.40; 100.36; 78.05; 77.87; 75.16; 75.02; 74.06; 73.71; 72.68; 71.77; 71.70; 69.52; 62.85; 62.69; 43.81; 42.65; 41.89; 21.82; 20.08; 19.97. LSI-MS: 1057.3 (13, $[3M+H]^+$), 493.1 (21), 398.1 (22, $[M+2Na]^+$), 397.1 (84, $[M+2Na-H]^+$), 375.1 (36, $[M+Na]^+$), 329.1 (21), 217.0 (23), 199.9 (26), 177.0 (26), 176.0 (100), 155.1 (30), 154.1 (41), 149.0 (41), 137.0 (35), 136.0 (39), 131.0 (26), 105.0 (32). Anal. calc. for $C_{14}H_{24}O_{10}\cdot H_2O$: C 45.41, H 7.08; found: C 45.64, H 6.90.

*α-[(α/β-*D*-Glucopyranosyl)oxy]-ω-hydroxyhexadecakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**13**). Compound **11** (400 mg, 198 μmol) was hydrogenated according to *GP IIIa* in CF_3CH_2OH (40 ml). FC (CH_2Cl_2/MeOH/H_2O 6:2.5:0.25) yielded **13** (238 mg, 77%), *β*-D/*α*-D 2.5:1. White solid. M.p. 128–129°. R_f 0.7 (CH_2Cl_2/MeOH/H_2O 6:2.5:0.25). $[\alpha]_D$ = +23.8 (*c* = 0.7, $CHCl_3$). IR ($CHCl_3$): 3502*w*, 2982*w*, 2933*w*, 1737*s*, 1450*w*, 1382*m*, 1303*m*, 1178*m*, 1134*m*, 1101*m*, 1057*s*, 979*w*. ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$): 5.30–5.24 (*m*, 15 CH); 4.97 (*d*, *J* = 4.03, H–C(1)(*α*)); 4.36 (*d*, *J* = 7.75, H–C(1)(*β*)); 4.30–4.26 (*m*, H–C(*β*)); 4.20–4.13 (*m*, H–C(*α*)); 3.86 (*dd*, *J* = 2.87, 11.98, H–C(6)(*β*)); 3.80 (*dd*, *J* = 3.17, 11.88, H–C(6)(*α*)); 3.78–3.67 (*m*, H–C(3)(*α*), H–C(5)(*α*), H–C(6)(*α*), H–C(6)(*β*)); 3.63 (*dd* (‘*t*’), *J* = 9.35, H–C(3)(*β*)); 3.43–3.27 (*m*, H–C(4)(*α*), H–C(4)(*β*), H–C(5)(*β*)); 3.21 (*dd* (‘*t*’), *J* = 9.02, H–C(2)(*β*)); 2.70–2.43 (*m*, 16 CH_2); 1.31 (*d*, *J* = 6.43, 2 Me); 1.29 (*d*, *J* = 6.33, 13 Me); 1.27 (*d*, *J* = 6.27, Me). ^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$): 169.99; 169.97; 169.93; 68.20; 68.16; 68.12; 41.13; 41.03; 19.88. LSI-MS: 1580.1 (79, $[M+Na]^+$), 1417.7 (11), 1396.6 (56), 1395.5 (75), 1285.7 (32), 1283.6 (56), 1282.5 (22), 1281.5 (21), 1193.6 (22), 1191.6 (40), 1190.5 (20), 1189.5 (20), 155.1 (100), 154.0 (43), 137.0 (33), 136.0 (34). Anal. calc. for $C_{70}H_{108}O_{38}\cdot H_2O$: C 53.36, H 7.03; found: C 53.35, H 7.25.

9. *Fluorescent HB Oligomers* **14**–**18**. *Benzyl (3*R*)-3-{{(3′*R*)-3′-{{[7-(Dimethylamino)-2-oxo-2*H*-1-benzopyran-4-yl]acetyl}oxy}butanoyl}oxy}butanoate* (**14**). To a suspension of 2-chloro-1-methylpyridinium iodide (290 mg, 0.97 mmol) in CH_2Cl_2 (5 ml) under Ar, a soln. of 7-(dimethylamino)-2-oxo-2*H*-1-benzopyran-4-acetic acid (200 mg, 0.81 mmol) and **1** (229 mg, 0.81 mmol) in CH_2Cl_2/MeCN 1:1 (6 ml) was added. After addition of Et_3N (270 μl, 1.93 mmol), stirring was continued for 12 h. The mixture was washed with 1N HCl, aq. sat. $NaHCO_3$, and NaCl soln. The org. phase was dried ($MgSO_4$) and evaporated. FC (Et_2O/pentane 3:1) yielded **14** (122 mg, 30%). Green oil. R_f 0.4 (Et_2O/pentane 3:1). $[\alpha]_D$ = +0.50 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 1736*s*, 1618*m*, 1532*w*, 1403*m*, 1277*w*, 1177*w*, 1059*w*. ^{1}H-NMR (300 MHz): 7.38 (*d*, *J*(5,6) = 8.95, H–C(5)); 7.37–7.32 (*m*, 5 arom. H); 6.61 (*dd*, *J*(5,6) = 8.95, *J*(6,8) = 2.56, H–C(6)); 6.55 (*d*, *J*(6,8) = 2.56, H–C(8)); 6.05 (*s*, H–C(3)); 5.33–5.22 (*m*, 2 CH); 5.08 (*s*, PhCH_2O); 3.65 (*s*, CH_2–C(4)); 3.05 (*s*, Me_2N); 2.66–2.37 (*m*, 2 CH_2); 1.28 (*d*, *J* = 6.36, Me); 1.24 (*d*, *J* = 6.39, Me). ^{13}C-NMR (75 MHz): 169.87; 169.15; 169.01; 168.29; 161.62; 155.98; 148.96; 135.71; 128.58; 128.33; 128.21; 125.36; 110.67; 109.01; 108.38; 98.35; 68.63; 67.77; 66.46; 40.88; 40.68; 40.64; 40.30; 19.79; 19.70. LSI-MS: 1021.2 (2, $[M+H]_2^+$), 1020.2 (7, $[2M+H]^+$), 1019.2 (12, $[M]_2^+$), 1018.1 (6, $[2M-H]^+$), 511.1 (33, $[M+2H]^+$), 510.1 (100, $[M+H]^+$), 509.1 (88, M^+), 508.1 (19, $[M-H]^+$), 203.1 (10).

*α-[[7-(Dimethylamino)-2-oxo-2*H*-1-benzopyran-4-yl]acetyl]-ω-(benzyloxy)hexadecakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**15**). As described for **14**, with 2-chloro-1-methylpyridinium iodide (97 mg, 0.32 mmol), CH_2Cl_2 (4 ml), 7-(dimethylamino)-2-oxo-2*H*-1-benzopyran-4-acetic acid (67 mg, 0.27 mmol), **2** (200 mg, 0.135 mmol), CH_2Cl_2/MeCN 1:1 (6 ml), and Et_3N (61 μl, 0.44 mmol; stirring for 18 h). FC (CH_2Cl_2/Et_2O 3:1) yielded **15** (49 mg, 21%). Yellow solid. M.p. 128–129°. R_f 0.6 (CH_2Cl_2/Et_2O 3:1). $[\alpha]_D$ = –0.55 (*c* = 1.1, $CHCl_3$). IR ($CHCl_3$): 3011*w*, 1738*s*, 1605*w*, 1384*w*, 1305*w*, 1261*w*, 1178*m*, 1100*w*, 1056*m*. ^{1}H-NMR (400 MHz): 7.39 (*d*, *J*(5,6) = 8.94, H–C(5)); 7.36–7.32 (*m*, 5 arom. H); 6.62 (*dd*, *J*(5,6) = 8.94, *J*(6,8) = 2.56, H–C(6)); 6.52 (*d*, *J*(6,8) = 2.56, H–C(8)); 6.05 (*s*, H–C(3)); 5.33–5.20 (*m*, 16 CH); 5.12 (*s*, PhCH_2O); 3.66 (*s*, CH_2–C(4)); 3.06 (*s*, Me_2N); 2.71–2.37 (*m*, 16 CH_2); 1.29–1.25 (*m*, 4 Me); 1.29 (*d*, *J* = 6.31, 2 Me); 1.27 (*d*, *J* = 6.31, 8 Me); 1.24 (*d*, *J* = 6.35, 2 Me). ^{13}C-NMR (100 MHz): 169.88; 169.12; 169.06; 168.29; 161.65; 155.95; 152.96; 148.31; 135.70; 128.58; 128.32; 125.36; 110.77; 109.00; 108.48; 98.34; 68.55; 67.67; 67.60; 66.46; 40.78; 40.68; 40.66; 40.60; 38.17; 19.79; 19.75; 19.70. LSI-MS: 1715.5 (32, $[M+H]^+$), 1714.6 (100, M^+), 1713.3 (23, $[M-H]^+$), 155.0 (12), 154.0 (11). Anal. calc. for $C_{84}H_{115}NO_{36}$: C 58.84, H 6.76, N 0.82; found: C 59.01, H 6.71, N 0.82.

*α-[[7-(Dimethylamino)-2-oxo-2*H*-1-benzopyran-4-yl]acetyl]-ω-hydroxyhexadecakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**16**). Compound **15** (30 mg, 18 μmol) was hydrogenated according to *GP IIIc* in 6 ml *N*,*N*-dimethylacetamide. FC (CH_2Cl_2/MeOH 10:1) yielded **16** (14 mg, 50%). Yellow solid. M.p. 115–116°. R_f 0.7 (CH_2Cl_2/MeOH 10:1). $[\alpha]_D$ = –1.15 (*c* = 0.9, $CHCl_3$). IR ($CHCl_3$): 2986*w*, 1738*s*, 1602*w*, 1306*w*, 1265*s*,

1178*m*, 1059*w*, 977*w*. ^{1}H-NMR (400 MHz): 7.45 (*d*, *J*(5,6) = 8.96, H–C(5)); 6.63 (*dd*, *J*(5,6) = 8.96, *J*(6,8) = 2.56, H–C(6)); 6.51 (*d*, *J*(6,8) = 2.55, H–C(8)); 6.08 (*s*, H–C(3)); 5.32–5.18 (*m*, 16 CH); 3.65 (*s*, CH_2–C(4)); 3.05 (*s*, Me_2N); 2.66–2.36 (*m*, 16 CH_2); 1.27–1.23 (*m*, 16 Me). ^{13}C-NMR (100 MHz): 170.24; 162.57; 156.03; 148.38; 135.53; 128.41; 128.08; 125.61; 110.36; 110.09; 108.65; 98.38; 68.61; 67.67; 66.56; 41.65; 40.87; 40.54; 40.17; 40.04; 19.79; 19.63. LSI-MS: 1655.5 (13), 1647.7 (12, $[M+Na]^+$), 1625.6 (100, $[M+H]^+$), 1624.7 (16, M^+), 1417.6 (22).

*Benzyl (3*R*)-3-{[(3*′R*)-3*′*-{[[5-(Dimethylamino)naphthalen-1-yl]sulfonyl}oxy}butanoyl]oxy}butanoate* (**17**). To a soln. of dansyl chloride (60 mg, 0.22 mmol) and **1** (41 mg, 0.15 mmol) in CH_2Cl_2 (15 ml) at 50° under Ar, a soln. of DMAP (15 mg, 0.15 mmol) in CH_2Cl_2 (5 ml) was added. After stirring was continued for 4 d, the soln. was washed with 1N HCl, aq. sat. $NaHCO_3$, and NaCl soln. The org. phase was dried ($MgSO_4$) and evaporated. FC (pentane/Et_2O 1:1) yielded **17** (11 mg, 15%). Yellow, oily solid. M.p. 133–134°. R_f 0.5 (pentane/Et_2O 1:1). IR ($CHCl_3$): 3038*w*, 1737*s*, 1573*w*, 1456*w*, 1358*w*, 1306*w*, 1175*w*, 1048*w*, 925*w*. ^{1}H-NMR (400 MHz): 8.58 (*d*, *J*(2,3) = 8.54, H–C(2)); 8.27 (*d*, *J*(3,4) = 7.31, H–C(4)); 8.22 (*d*, *J*(7,8) = 9.61, H–C(8)); 7.58–7.51 (*m*, H–C(3), H–C(7)); 7.37-7.31 (*m*, 5 arom. H); 7.17 (*d*, *J*(6,7) = 7.58, H–C(6)); 5.14–5.09 (*m*, CH); 5.09 (*s*, PhCH_2O); 4.94–4.89 (*m*, CH); 2.88 (*s*, Me_2N); 2.57, 2.50 (*AB* of *ABX*, J_{AB} = 15.69, J_{AX} = 7.31, J_{BX} = 5.86); 2.64, 2.42 (*AB* of *ABX*, J_{AB} = 15.71, J_{AX} = 7.47, J_{BX} = 5.72); 1.21 (*d*, *J* = 6.41, Me); 1.20 (*d*, *J* = 6.39, Me). ^{13}C-NMR (100 MHz): 169.82; 168.33; 151.77; 135.64; 132.35; 131.45; 129.88; 129.82; 128.59; 128.54; 128.37; 128.34; 123.04; 119.50; 115.44; 67.95; 66.48; 45.39; 41.55; 40.46; 20.60; 19.64. LSI-MS: 1028.0 (8, $[2M+H]^+$), 1027.0 (15, $[M]_2^+$), 1026.0 (5, $[2M-H]^+$), 514.9 (41, $[M+2H]^+$), 513.9 (91, $[M+H]^+$), 512.9 (100, M^+), 511.9 (22, $[M-H]^+$), 251.9 (25), 250.9 (66), 249.9 (17), 170.0 (25), 168.0 (16), 154.0 (26), 137.0 (17), 136.0 (22). Anal. calc. for $C_{27}H_{31}NO_7S$: C 63.14, H 6.08, N 2.72; found: C 63.16, H 6.25, N 2.76.

*α-{[5-(Dimethylamino)naphthalen-1-yl]sulfonyl}-ω-(benzyloxy)hexadecakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**18**). As described for **17**, with dansyl chloride (188 mg, 0.7 mmol), **2** (520 mg, 0.35 mmol), CH_2Cl_2 (60 ml), DMAP (44 mg, 0.37 mmol) in CH_2Cl_2 (15 ml), stirring for 2 d. FC (CH_2Cl_2/Et_2O 3:1) yielded **18** (67 mg, 12%). Yellow solid. M.p. 152–154°. R_f 0.8 (CH_2Cl_2/Et_2O 3:1). $[\alpha]_D$ = –1.07 (*c* = 0.8, $CHCl_3$). IR ($CHCl_3$): 3038*w*, 2985*w*, 1737*s*, 1580*w*, 1450*w*, 1382*w*, 1358*w*, 1306*m*, 1176*m*, 1129*w*, 1047*w*, 938*w*. ^{1}H-NMR (400 MHz): 8.57 (*d*, *J*(2,3) = 8.53, H–C(2)); 8.30 (*d*, *J*(3,4) = 7.28, H–C(4)); 8.23 (*d*, *J*(7,8) = 9.61, H–C(8)); 7.59–7.51 (*m*, H–C(3), H–C(7)); 7.36–7.32 (*m*, 5 arom. H); 7.16 (*d*, *J*(6,7) = 7.54, H–C(6)); 5.31–5.15 (*m*, 15 CH); 5.08 (*s*, PhCH_2O); 4.95–4.90 (*m*, CH); 2.87 (*s*, Me_2N); 2.73–2.36 (*m*, 16 CH_2); 1.28–1.20 (*m*, 16 Me). ^{13}C-NMR (100 MHz): 172.00; 169.20; 169.12; 169.06; 168.28; 161.63; 155.95; 153.01; 148.41; 135.72; 132.45; 131.44; 129.89; 129.87; 128.60; 128.53; 128.39; 128.33; 122.98; 120.08; 115.43; 68.45; 67.65; 67.62; 66.48; 45.41; 41.53; 40.76; 40.68; 40.65; 40.62; 19.79; 19.74; 19.64. LSI-MS: 1719.9 (25, $[M+H]^+$), 1718.8 (100, M^+), 155.0 (18).

10. *Glycine-OHB Conjugates* **19**–**21**. *Benzyl (3*R*)-3-{[(3*′R*)-3*′*-{[2-{[(*tert*-Butoxy)carbonyl]amino}acetyl]-oxy}butanoyl]oxy}butanoate* (**19**). Compound **1** (700 mg, 2.50 mmol) was transformed according to *GP II* with commercially available *N*-[(*tert*-butoxy)carbonyl]glycine (500 mg, 2.86 mmol) in CH_2Cl_2 (120 ml). FC (CH_2Cl_2/Et_2O 4:1) yielded **19** (970 mg, 89%). Colorless oil. R_f 0.8 (CH_2Cl_2/Et_2O 4:1). $[\alpha]_D$ = +0.81 (*c* = 1.1, $CHCl_3$). IR ($CHCl_3$): 2985*w*, 1737*s*, 1508*w*, 1456*w*, 1368*w*, 1262*w*, 1107*m*, 1102*w*, 1058*m*, 974*w*. ^{1}H-NMR (400 MHz): 7.38–7.31 (*m*, 5 arom. H); 5.34–5.26 (*m*, 2 CH); 5.12 (*s*, PhCH_2O); 5.06 (br., NH); 3.86–3.84 (*m*, CH_2NH); 2.68, 2.56 (*AB* of *ABX*, J_{AB} = 15.63, J_{AX} = 7.80, J_{BX} = 5.18); 2.55, 2.42 (*AB* of *ABX*, J_{AB} = 15.57, J_{AX} = 7.94, J_{BX} = 5.60); 1.45 (*s*, *t*-Bu); 1.28 (*d*, *J* = 6.34, Me); 1.27 (*d*, *J* = 6.32, Me). ^{13}C-NMR (100 MHz): 169.92; 169.52; 160.11; 155.64; 135.67; 128.57; 128.34; 128.33; 79.88; 68.34; 67.73; 66.51; 42.51; 40.77; 40.65; 28.30; 19.81; 19.77. LSI-MS: 876.2 (11, $[2M+H]^+$), 875.2 (22, $[M]_2^+$), 438.1 (16, $[M+H]^+$), 382.0 (23), 339.1 (29), 338.1 (100), 154.0 (11), 137.0 (16). Anal. calc. for $C_{22}H_{31}N_8$: C 60.40, H 7.14, N 3.20; found: C 60.24, H 7.19, N 3.47.

*α-{2-{[(*tert*-Butoxy)carbonyl]amino}acetyl}-ω-(benzyloxy)hexadecakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**20**). Compound **2** (2.0 g, 1.35 mmol) was transformed according to *GP II* with commercially available *N*-[(*tert*-butoxy)carbonyl]glycine (350 mg, 2.0 mmol) in CH_2Cl_2 (90 ml). FC (CH_2Cl_2/Et_2O 5:1) yielded **20** (1.82 g, 82%). White solid. M.p. 128–129°. R_f 0.5 (CH_2Cl_2/Et_2O 5:1). $[\alpha]_D$ = –0.31 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 2985*w*, 1737*s*, 1504*w*, 1458*w*, 1382*w*, 1306*m*, 1266*w*, 1178*m*, 1136*w*, 1102*w*, 1060*m*, 978*w*. ^{1}H-NMR (400 MHz): 7.37–7.31 (*m*, 5 arom. H); 5.35–5.30 (br., NH); 5.29–5.20 (*m*, 16 CH); 5.12 (*s*, PhCH_2O); 3.88–3.85 (*m*, CH_2NH); 2.71–2.37 (*m*, 16 CH_2); 1.45 (*s*, *t*-Bu); 1.31 (*d*, *J* = 6.33, 2 Me); 1.28 (*d*, *J* = 5.70, 2 Me); 1.28 (*d*, *J* = 6.31, 8 Me); 1.27 (*d*, *J* = 6.13, 2 Me); 1.25 (*d*, *J* = 6.34, 2 Me). ^{13}C-NMR (100 MHz): 169.20; 169.15; 128.61; 128.35; 68.38; 67.70; 67.62; 66.49; 40.82M 40.69; 28.34; 19.84; 19.82; 19.76; 19.74. LSI-MS: 1664.4 (3, $[M+Na]^+$), 1642.3 (1, $[M+H]^+$), 1542.4 (100, $[M-Boc]^+$), 155.0 (27). Anal. calc. for $C_{78}H_{115}NO_{36}$: C 57.03, H 7.06, N 0.85; found: C 56.99, H 7.29, N 1.04.

*α-{2-{[(*tert*-Butoxy)carbonyl]amino}acetyl}-ω-(benzyloxy)dotriacontakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**21**). Compound **3** (840 mg, 0.29 mmol) was transformed according to *GP II* with commercially available *N*-[(*tert*-butoxy)carbonyl]glycine (77 mg, 0.44 mmol) in CH_2Cl_2 (60 ml). FC (CH_2Cl_2/Et_2O 2:1) yielded **21** (670 mg, 76%). White solid. M.p. 125–126°. R_f 0.2 (CH_2Cl_2/Et_2O 2:1). $[\alpha]_D = +1.45$ ($c = 1.1$, $CHCl_3$). IR ($CHCl_3$): 2987*w*, 1739*s*, 1458*w*, 1383*w*, 1306*m*, 1262*w*, 1178*m*, 1135*w*, 1058*m*, 978*w*. ^{1}H-NMR (500 MHz): 7.37–7.32 (*m*, 5 arom. H); 5.45–5.42 (br., NH); 5.28–5.20 (*m*, 32 CH); 5.12 (*s*, PhCH_2O); 3.89–3.85 (*m*, CH_2NH); 2.71–2.38 (*m*, 32 CH_2); 1.45 (*s*, *t*-Bu); 1.31 (*d*, $J = 6.34$, Me); 1.28 (*d*, $J = 6.32$, 2 Me); 1.27 (*d*, $J = 6.32$, 25 Me); 1.27 (*d*, $J = 6.03$, 2 Me); 1.24 (*d*, $J = 6.31$, 2 Me). ^{13}C-NMR (125 MHz): 169.91; 169.20; 169.17; 169.15; 155.69; 135.72; 128.61; 128.35; 68.38; 67.78; 67.70; 67.63; 67.46; 66.49; 40.81; 40.68; 28.33; 19.91; 19.84; 19.82; 19.78; 19.74; 19.61; 18.04. MALDI-MS: 3043.1 ($[M + Na]^+$). Anal. calc. for $C_{124}H_{211}NO_{68}$: C 56.47, H 7.04, N 0.46; found: C 56.22, H 7.21, N 0.51.

11. *Fluorescent Glycine-OHB Conjugates* **22**–**26**. *Benzyl (3*R*)-3-{{(3′*R*)-3′-{{2-{[2-[7-(Dimethylamino)-2-oxo-2*H*-1-benzopyran-4-yl]acetyl}amino}acetyl}oxy}butanoyl}oxy}butanoate* (**22**). Boc deprotection of **19** (250 mg, 0.58 mmol) in CH_2Cl_2 (5 ml) according to *GP IV*. The obtained crude trifluoroacetate was treated with 7-(dimethylamino)-2-oxo-2*H*-1-benzopyran-4-acetic acid (143 mg, 0.58 mmol) according to *GP I*. FC (CH_2Cl_2/Et_2O 2:1) yielded **22** (262 mg, 80%). Green oil. R_f 0.3 (CH_2Cl_2/Et_2O 2:1). $[\alpha]_D = -14.0$ ($c = 0.9$, $CHCl_3$). IR ($CHCl_3$): 3011*w*, 2988*w*, 1738*s*, 1603*w*, 1384*w*, 1306*m*, 1265*s*, 1177*m*, 1135*w*, 1101*w*, 1059*w*, 976*w*. ^{1}H-NMR (400 MHz): 7.47 (*d*, $J(5,6) = 8.98$, H–C(5)); 7.37–7.32 (*m*, 5 arom. H); 6.61 (*dd*, $J(5,6) = 8.98$, $J(6,8) = 2.56$, H–C(6)); 6.49 (*d*, $J(6,8) = 2.56$, H–C(8)); 6.35 (*dd*, $J = 5.43$, 1.17, NH); 6.08 (*s*, H–C(3)); 5.33–5.20 (*m*, 2 CH); 5.07 (*s*, PhCH_2O); 3.97 (*dd*, $J = 5.43$, 1.17, CH_2NH); 3.66 (*s*, CH_2CONH); 3.04 (*s*, Me_2N); 2.64–2.37 (*m*, 2 CH_2); 1.27 (*d*, $J = 6.37$, Me); 1.24 (*d*, $J = 6.36$, Me). ^{13}C-NMR (100 MHz): 170.10; 169.18; 168.55; 168.03; 161.59; 156.03; 149.15; 135.57; 128.57; 128.33; 128.26; 125.63; 110.58; 109.16; 108.31; 98.26; 68.63; 67.78; 66.54; 41.62; 40.71; 40.65; 40.23; 40.06; 19.84; 19.72. LSI-MS: 1134.2 (3, $[M + H]_2^+$), 1133.2 (6, $[2M + H]^+$), 1332.1 (3, $[M]_2^+$), 568.1 (34, $[M + 2H]^+$), 567.1 (100, $[M + H]^+$), 566.1 (82, M^+), 565.1 (25, $[M - H]^+$), 154.0 (21), 136.0 (27). Anal. calc. for $C_{30}H_{34}N_2O_9$: C 63.59, H 6.05, N 4.94; found: C 63.59, H 6.30, N 4.92.

*α-{2-{[2-[7-(Dimethylamino)-2-oxo-2*H*-1-benzopyran-4-yl]acetyl}amino}acetyl}-ω-(benzyloxy)hexadecakis-[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**23**). Boc deprotection of **20** (600 mg, 0.36 mmol) in CH_2Cl_2 (5 ml) according to *GP IV*. The obtained crude trifluoroacetate was reacted according to *GP I* with 7-(dimethylamino)-2-oxo-2*H*-1-benzopyran-4-acetic acid (99 mg, 0.4 mmol). FC (CH_2Cl_2/Et_2O/MeOH 8:2:0.2) yielded **23** (557 mg, 86%). Yellow solid. M.p. 124–125°. R_f 0.5 (CH_2Cl_2/Et_2O/MeOH 8:2:0.2). $[\alpha]_D = -2.5$ ($c = 0.8$, $CHCl_3$). IR ($CHCl_3$): 2985*w*, 1737*s*, 1508*w*, 1456*w*, 1368*w*, 1262*w*, 1107*m*, 1102*w*, 1058*m*, 974*w*. ^{1}H-NMR (400 MHz): 7.44 (*d*, $J(5,6) = 8.97$, H–C(5)); 7.38–7.30 (*m*, 5 arom. H); 6.60 (*dd*, $J(5,6) = 8.97$, $J(6,8) = 2.57$, H–C(6)); 6.50 (*d*, $J(6,8) = 2.56$, H–C(8)); 6.34 (*dd*, $J = 5.43$, 1.16, NH); 6.08 (*s*, H–C(3)); 5.33–5.20 (*m*, 16 CH); 5.07 (*s*, PhCH_2O); 3.98 (*dd*, $J = 5.43$, 1.16, CH_2NH); 3.64 (*s*, CH_2CONH); 3.05 (*s*, Me_2N); 2.64–2.37 (*m*, 16 CH_2); 1.27–1.22 (*m*, 16 Me). ^{13}C-NMR (100 MHz): 170.11; 169.18; 168.57; 168.20; 161.62; 156.03; 149.14; 135.59; 128.55; 128.32; 128.27; 125.62; 110.58; 109.18; 109.03; 99.26; 68.62; 67.72; 66.43; 41.62; 40.71; 40.60; 40.33; 40.07; 19.84; 19.73. LSI-MS: 1772.6 (28, $[M + H]^+$), 1771.5 (100, M^+), 1770.5 (22, $[M - H]^+$). Anal. calc. for $C_{86}H_{118}N_2O_{37}$: C 58.03, H 6.71, N 1.58; found: C 58.08, H 6.61, N 1.64.

*α-{2-{[2-[7-(Dimethylamino)-2-oxo-2*H*-1-benzopyran-4-yl]acetyl}amino}acetyl}-ω-(benzyloxy)dotriacontakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**24**). Boc deprotection of **21** (80 mg, 26 μmol) in CH_2Cl_2 (2 ml) according to *GP IV*. The obtained crude trifluoroacetate was reacted according to *GP I* with 7-(dimethylamino)-2-oxo-2*H*-1-benzopyran-4-acetic acid (13 mg, 50 μmol). FC (CH_2Cl_2/Et_2O/MeOH 10:5:0.1) yielded **24** (57 mg, 70%). Yellow solid. M.p. 125–126°. R_f 0.6 (CH_2Cl_2/Et_2O/MeOH 10:5:0.1). $[\alpha]_D = -0.71$ ($c = 0.7$, $CHCl_3$). IR ($CHCl_3$): 3032*w*, 1738*s*, 1602*w*, 1383*w*, 1305*w*, 1178*m*, 1101*w*, 1059*m*, 974*w*. ^{1}H-NMR (500 MHz): 7.50 (*d*, $J(5,6) = 9.00$, H–C(5)); 7.46–7.36 (*m*, 5 arom. H); 6.63 (*dd*, $J(5,6) = 9.00$, $J(6,8) = 2.54$, H–C(6)); 6.52 (*d*, $J(6,8) = 2.54$, H–C(8)); 6.50–6.48 (*m*, NH); 6.10 (*s*, H–C(3)); 5.31–5.20 (*m*, 32 CH); 5.07 (*s*, PhCH_2O); 3.98 (*dd*, $J = 5.49$, CH_2NH); 3.69 (*s*, CH_2CONH); 3.06 (*s*, Me_2N); 2.71–2.38 (*m*, 32 CH_2); 1.29–1.24 (*m*, 32 Me). ^{13}C-NMR (100 MHz): 169.91; 169.43; 169.28; 169.24; 169.15; 168.62; 168.15; 161.60; 156.08; 153.13; 149.29; 135.72; 128.61; 128.35; 125.75; 110.64; 109.18; 108.45; 98.31; 68.67; 67.71; 67.67; 67.63; 66.48; 41.66; 40.93; 40.81; 40.68; 40.21; 40.13; 19.89; 19.78; 19.74. MALDI-MS: 3170.1 ($[M + Na]^+$). Anal. calc. for $C_{150}H_{214}N_2O_{69} \cdot 12\,H_2O$: C 53.49, H 7.07, N 0.83; found: C 53.50, H 7.05, N 0.78.

*α-{2-{[2-[7-(Dimethylamino)-2-oxo-2*H*-1-benzopyran-4-yl]acetyl}amino}acetyl}-ω-hydroxyhexadecakis-[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**25**). Compound **23** (50 mg, 28 μmol) was hydrogenated according to *GP IIIc* in DMF (4 ml). FC (CH_2Cl_2/MeOH 20:1) yielded **25** (24 mg, 50%). Yellow solid. M.p. 115–116°. R_f 0.1 (CH_2Cl_2/MeOH 20:1). $[\alpha]_D = -1.13$ ($c = 1.2$, $CHCl_3$). UV/VIS (CF_3CH_2OH): 215 (1600), 378 (15000). IR ($CHCl_3$): 3011*w*, 2987*w*, 1738*s*, 1603*w*, 1383*w*, 1306*m*, 1265*s*, 1177*m*, 1136*w*, 1101*w*, 1059*w*, 976*w*. ^{1}H-NMR

(400 MHz): 7.47 (*d*, *J*(5,6) = 8.97, H–C(5)); 6.63 (*dd*, *J*(5,6) = 8.99, *J*(6,8) = 2.55, H–C(6)); 6.50 (*d*, *J*(6,8) = 2.56, H–C(8)); 6.38 (*dd*, *J* = 5.43, 1.17, NH); 6.10 (*s*, H–C(3)); 5.33–5.18 (*m*, 16 CH); 3.95 (*dd*, *J* = 5.41, 1.18, CH_2NH); 3.64 (*s*, CH_2CONH); 3.04 (*s*, Me_2N); 2.64–2.37 (*m*, 16 CH_2); 1.27–1.22 (*m*, 16 Me). ^{13}C-NMR (100 MHz): 170.14; 162.59; 156.02; 148.35; 135.55; 128.48; 128.36; 128.16; 125.63; 110.38; 110.16; 108.61; 98.36; 68.63; 67.68; 66.56; 41.67; 40.89; 40.55; 40.20; 40.03; 19.82; 19.62. LSI-MS: 1647.7 (12, $[M+Na]^+$), 1625.6 (100, $[M+H]^+$), 1624.7 (16, M^+), 1417.6 (22), 155.1 (77), 154.1 (29), 137.1 (26), 136.0 (26).

*α-[2-{[2-[7-(Dimethylamino)-2-oxo-2*H*-1-benzopyran-4-yl]acetyl}amino}acetyl]-ω-hydroxydotriacontakis-[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**26**). Compound **24** (40 mg, 13 μmol) was hydrogenated according to *GP IIIb* in CF_3CH_2OH (5 ml). FC (CH_2Cl_2/Et_2O/MeOH 10:3:0.2) yielded **26** (22 mg, 55%). Yellow solid. M.p. 123–124°. R_f 0.3 (CH_2Cl_2/Et_2O/MeOH 10:3:0.2). $[\alpha]_D = -2.34$ (*c* = 0.9, $CHCl_3$). UV/VIS (CF_3CH_2OH): 214 (3300), 376 (15000). IR ($CHCl_3$): 3012*w*, 2987*w*, 1739*s*, 1600*w*, 1384*w*, 1306*m*, 1264*s*, 1178*m*, 1135*w*, 1102*w*, 1061*w*, 976*w*. ^{1}H-NMR (500 MHz): 7.46 (*d*, *J*(5,6) = 8.98, H–C(5)); 6.62 (*dd*, *J*(5,6) = 8.99, *J*(6,8) = 2.54, H–C(6)); 6.50 (*d*, *J*(6,8) = 2.55, H–C(8)); 6.37 (*dd*, *J* = 5.44, 1.20, NH); 6.09 (*s*, H–C(3)); 5.34–5.20 (*m*, 32 CH); 3.95 (*dd*, *J* = 5.42, 1.20, CH_2NH); 3.64 (*s*, CH_2CONH); 3.03 (*s*, Me_2N); 2.65–2.36 (*m*, 32 CH_2); 1.27–1.21 (*m*, 32 Me). ^{13}C-NMR (125 MHz): 170.23; 162.62; 156.03; 148.48; 135.65; 128.49; 128.37; 128.16; 125.64; 110.41; 110.11; 108.59; 98.37; 68.64; 67.74; 67.69; 66.66; 41.68; 40.92; 40.45; 40.19; 40.02; 19.83; 19.60. MALDI-MS: 3087.5 ($[M+Na]^+$).

12. *Biotin-OHB Conjugates* **27**–**30**. *Benzyl (3*R*)-3-{[(3′*R*)-3′-{[5-[(3a*S*,4*S*,6a*R*)-Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}oxy}butanoyl}oxy}butanoate (***27***)*. Compound **1** (560 mg, 2 mmol) was transformed according to *GP II* with commercial (+)-biotin (537 mg, 2.2 mmol) in CH_2Cl_2/DMF 3:5 (80 ml). FC (AcOEt/MeOH 20:1) yielded **27** (197 mg, 19%). Colorless oil. R_f 0.4 (AcOEt/MeOH 20:1). $[\alpha]_D = +15.92$ (*c* = 0.7, $CHCl_3$). IR ($CHCl_3$): 3468*w*, 3032*w*, 2934*w*, 1731*s*, 1456*m*, 1383*w*, 1304*m*, 1266*m*, 1177*m*, 1137*w*, 1101*w*, 1060*w*, , 976*w*. ^{1}H-NMR (400 MHz): 7.36–7.31 (*m*, 5 arom. H); 5.68–5.64 (br., 2 NH); 5.32–5.20 (*m*, 2 CH); 5.12 (*s*, PhCH_2O); 4.50–4.46 (*m*, H–C(3a)); 4.31–4.28 (*m*, H–C(6a)); 3.16–3.11 (*m*, H–C(4)); 2.89 (*dd*, *J*(6,6′) = 12.79, *J*(6,6a) = 5.18, H–C(6)); 2.72 (*d*, *J*(6,6′) = 12.78, H′–C(6)); 2.68, 2.56 (*AB* of *ABX*, J_{AB} = 15.64, J_{AX} = 7.63, J_{BX} = 5.43); 2.53, 2.41 (*AB* of *ABX*, J_{AB} = 15.48, J_{AX} = 7.61, J_{BX} = 5.49); 2.28 (*t*, *J* = 7.64, CH_2CO); 1.72–1.62 (*m*, 2 CH_2); 1.47–1.40 (*m*, CH_2); 1.29 (*d*, *J* = 6.33, Me); 1.25 (*d*, *J* = 6.33, Me). ^{13}C-NMR (100 MHz): 172.76; 170.00; 169.40; 163.39; 135.73; 128.61; 128.33; 67.72; 67.12; 66.50; 61.92; 60.10; 55.37; 40.93; 40.72; 40.55; 34.04; 28.28; 28.23; 24.78; 19.87; 19.84. LSI-MS: 1015.5 (4, $[M+H]_2^+$), 1014.4 (11, $[2M+H]^+$), 1013.4 (21, $[M]_2^+$), 529.1 (7, $[M+Na]^+$), 508.1 (44, $[M+2H]^+$), 507.1 (100, $[M+H]^+$), 431.2 (12). Anal. calc. for $C_{25}H_{34}N_2O_7S$: C 59.27, H 6.76, N 5.53; found: C 59.30, H 6.61, N 5.63.

*α-[5-[(3a*S*,4*S*,6a*R*)-Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl]-ω-(benzyloxy)hexadecakis-[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**28**). Compound **2** (700 mg, 0.47 mmol) was transformed according to *GP II* with commercial (+)-biotin (122 mg, 0.5 mmol) in CH_2Cl_2/DMF 1:2 (60 ml). FC (AcOEt/MeOH 20:1) yielded **28** (90 mg, 11%). White solid. M.p. 129–130°. R_f 0.6 (AcOEt/MeOH 20:1). $[\alpha]_D = +17.05$ (*c* = 0.7, $CHCl_3$). IR ($CHCl_3$): 3011*m*, 2935*w*, 1737*s*, 1602*w*, 1522*w*, 1449*w*, 1426*w*, 1383*w*, 1305*m*, 1178*m*, 1135*w*, 1100*w*, 1058*m*, 979*w*, 928*w*. ^{1}H-NMR (400 MHz): 7.36–7.34 (*m*, 5 arom. H); 5.28–5.20 (*m*, 16 CH); 5.12 (*s*, PhCH_2O); 4.98–4.96 (br., NH); 4.66–4.65 (br., NH); 4.54–4.50 (*m*, H–C(3a)); 4.35–4.32 (*m*, H–C(6a)); 3.19–3.15 (*m*, H–C(4)); 2.93 (*dd*, *J*(6,6′) = 12.80, *J*(6,6a) = 5.04, H–C(6)); 2.73 (*d*, *J*(6,6′) = 12.79, H′–C(6)); 2.68–2.41 (*m*, 16 CH_2); 2.29 (*t*, *J* = 7.33, CH_2CO); 1.71–1.64 (*m*, 2 CH_2); 1.48–1.42 (*m*, CH_2); 1.28 (*d*, *J* = 5.72, 2 Me); 1.27 (*d*, *J* = 6.30, 8 Me); 1.27 (*d*, *J* = 6.32, 4 Me); 1.24 (*d*, *J* = 6.33, 2 Me). ^{13}C-NMR (100 MHz): 172.62; 169.34; 169.13; 128.58; 128.32; 67.67; 67.60; 67.15; 66.46; 61.79; 60.02; 55.15; 40.88; 40.77; 40.64; 40.52; 33.94; 28.19; 24.69; 19.89; 19.75; 19.70. LSI-MS: 1818.4 (23), 1713.8 (14, $[M+H]^+$), 1712.2 (100, M^+), 155.1 (18), 154.1 (17), 137.0 (13), 136.0 (14). Anal. calc. for $C_{81}H_{118}N_2O_{35}S$: C 56.83, H 6.95, N 1.64, S 1.87; found: C 57.09, H 6.78, N 1.79, S 2.07.

*(3*R*)-3-{[(3′*R*)-3′-{[5-[(3a*S*,4*S*,6a*R*)-Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}oxy}butanoyl}oxy}butanoic Acid* (**29**). Compound **27** (30 mg, 55 μmol) was hydrogenated according to *GP IIIa* in CF_3CH_2OH (5 ml). FC (CH_2Cl_2/MeOH 2:1) yielded **29** (24 mg, 81%). Colorless oil. R_f 0.7 (CH_2Cl_2/MeOH 2:1). IR (KBr): 3276*w*, 2932*w*, 1735*s*, 1702*s*, 1448*w*, 1386*w*, 1302*w*, 1265*m*, 1178*w*, 1061*w*, 970*w*. ^{1}H-NMR (300 MHz, CD_3OD): 5.30–5.22 (*m*, 2 CH); 4.53–4.50 (*m*, H–C(3a)); 4.34–4.31 (*m*, H–C(6a)); 3.19–3.14 (*m*, H–C(4)); 2.95 (*dd*, *J*(6,6′) = 12.82, *J*(6,6a) = 5.03, H–C(6)); 2.74 (*d*, *J*(6,6′) = 12.81, H′–C(6)); 2.61, 2.58 (*AB of ABX*, J_{AB} = 15.63, J_{AX} = 7.81, J_{BX} = 5.51); 2.57, 2.47 (*AB* of *ABX*, J_{AB} = 15.41, J_{AX} = 7.79, J_{BX} = 5.40); 2.28 (*t*, *J* = 7.35, CH_2CO); 1.74–1.64 (*m*, 2 CH_2); 1.45–1.39 (*m*, CH_2); 1.28 (*d*, *J* = 5.98, Me); 1.25 (*d*, *J* = 6.34, Me). ^{13}C-NMR (75 MHz, CD_3OD): 171.24; 170.54; 169.82; 169.81; 163.30; 61.64; 56.94; 42.26; 42.12; 41.81; 41.12; 36.38; 29.61; 29.42; 26.66; 20.09; 20. LSI-MS: 455.2 (7, $[M+K]^+$), 439.7 (13, $[M+Na]^+$), 416.7 (100, $[M+H]^+$).

*α-[5-[(3a*S*,4*S*,6a*R*)-Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl]-ω-hydroxyhexadecakis-[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**30**). Compound **28** (35 mg, 20 μmol) was hydrogenated according to *GP IIIa* in CF_3CH_2OH (5 ml). FC (CH_2Cl_2/MeOH 5:1) yielded **30** (27 mg, 82%). White solid. M.p. 138–139°. R_f 0.7 (CH_2Cl_2/MeOH 5:1). $[\alpha]_D = -2.67$ ($c = 0.9$, MeOH). IR (KBr): 3277*w*, 2934*w*, 1737*s*, 1698*s*, 1452*w*, 1382*w*, 1302*w*, 1264*m*, 1176*w*, 1060*w*, 970*w*. ^{1}H-NMR (300 MHz, CD_3OD): 5.28–5.19 (*m*, 16 CH); 4.55–4.50 (*m*, H–C(3a)); 4.35–4.31 (*m*, H–C(6a)); 3.19–3.15 (*m*, H–C(4)); 2.94 (*dd*, *J*(6,6′) = 12.81, *J*(6,6a) = 5.02, H–C(6)); 2.76 (*d*, *J*(6,6′) = 12.79, H′–C(6)); 2.65–2.41 (*m*, 16 CH_2); 2.28 (*t*, *J* = 7.36, CH_2CO); 1.73–1.64 (*m*, 2 CH_2); 1.47–1.40 (*m*, CH_2); 1.28 (*d*, *J* = 5.92, 2 Me); 1.27 (*d*, *J* = 6.31, 8 Me); 1.27 (*d*, *J* = 6.32, 4 Me); 1.23 (*d*, *J* = 6.33, 2 Me). ^{13}C-NMR (75 MHz, CD_3OD): 169.25; 169.20; 169.16; 169.09; 68.60; 67.69; 67.66; 67.63; 67.60; 66.43; 61.68; 60.12; 55.29; 41.19; 40.76; 40.71; 40.65; 40.43; 35.52; 28.10; 28.01; 25.45; 19.81; 19.74; 19.05. LSI-MS: 1728.4 (78), 1660.5 (2, $[M+K]^+$), 1644.2 (11, $[M+Na]^+$), 1622.4 (100, $[M+H]^+$), 1592.3 (34).

13. *'Biotinylated' Glycine-OHB Conjugates* **31**–**34**. *Benzyl (3*R*)-3-{{(3′*R*)-3′-{{2-{{5-[(3a*S*,4*S*,6a*R*)-Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}amino}acetyl}oxy}butanoyl}oxy}butanoate* (**31**). Boc deprotection of **19** (400 mg, 0.92 mmol) in CH_2Cl_2 (2 ml) according to *GP IV*. The obtained crude trifluoroacetate was treated with (+)-biotin (225 mg, 0.92 mmol) according to *GP I*. FC (AcOEt/MeOH 7:1) yielded **31** (440 mg, 85%). White, gelatinous solid. M.p. 94–95°. R_f 0.4 (AcOEt/MeOH 7:1). $[\alpha]_D = +3.56$ ($c = 0.9$, $CHCl_3$). IR ($CHCl_3$): 3278*w*, 2936*w*, 1738*s*, 1700*s*, 1457*w*, 1382*w*, 1304*w*, 1265*m*, 1177*w*, 1057*w*, 974*w*. ^{1}H-NMR (400 MHz): 7.37–7.32 (*m*, 5 arom. H); 6.91–6.90 (br., NH); 6.44–6.43 (br., NH); 5.38–5.36 (br., NH); 5.32–5.24 (*m*, 2 CH); 5.12 (*s*, PhCH_2O); 4.51–4.48 (*m*, H–C(3a)); 4.32–4.29 (*m*, H–C(6a)); 4.06 (*dd*, *J* = 18.16, 5.87, 1 H, CH_2NH); 3.91 (*dd*, *J* = 18.16, 5.18, 1 H, CH_2NH); 3.15–3.11 (*m*, H–C(4)); 2.90 (*dd*, *J*(6,6′) = 12.80, *J*(6,6a) = 4.97, H–C(6)); 2.72 (*d*, *J*(6,6′) = 12.80, H′–C(6)); 2.66, 2.57 (*AB* of *ABX*, $J_{AB} = 15.69$, $J_{AX} = 7.79$, $J_{BX} = 5.29$); 2.55, 2.43 (*AB* of *ABX*, $J_{AB} = 15.69$, $J_{AX} = 7.60$, $J_{BX} = 5.49$); 2.30–2.26 (*m*, CH_2CO); 1.77–1.65 (*m*, 2 CH_2); 1.47–1.41 (*m*, CH_2); 1.29 (*d*, *J* = 6.34, Me); 1.28 (*d*, *J* = 6.35, Me). ^{13}C-NMR (100 MHz): 173.52; 170.05; 169.97; 169.20; 163.89; 135.66; 128.60; 128.36; 128.31; 68.54; 67.79; 66.52; 61.68; 60.18; 55.45; 41.20; 40.70; 40.66; 40.47; 35.54; 28.07; 25.47; 19.82; 19.78. LSI-MS: 1128.4 (15, $[M+H]_2^+$), 1127.4 (25, $[2M+H]^+$), 587.1 (14, $[M+Na+H]^+$), 586.1 (41, $[M+Na]^+$), 565.1 (40, $[M+2H]^+$), 564.1 (100, $[M+H]^+$), 227.0 (14). Anal. calc. for $C_{27}H_{37}N_3O_8S$: C 57.53, H 6.62, N 7.45, S 5.69; found: C 57.40, H 6.47, N 7.53, S 5.66.

*α-[2-{{5-[(3a*S*,4*S*,6a*R*)-Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}amino}acetyl]-ω-(benzyl-oxy)hexadecakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**32**). Boc deprotection of **20** (600 mg, 0.37 mmol) in CH_2Cl_2 (5 ml) according to *GP IV*. The obtained crude trifluoroacetate was treated with (+)-biotin (111 mg, 0.40 mmol) according to *GP I*. FC (AcOEt/MeOH 8:1) yielded **32** (505 mg, 78%). White solid. M.p. 133–134°. R_f 0.3 (AcOEt/MeOH 8:1). $[\alpha]_D = +1.04$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 1737*s*, 1458*w*, 1382*w*, 1306*w*, 1267*w*, 1178*w*, 1135*w*, 1101*w*, 1060*w*, 978*w*. ^{1}H-NMR (400 MHz): 7.37–7.32 (*m*, 5 arom. H); 6.77–6.75 (br., NH); 6.03–6.01 (br., NH); 5.36–5.32 (br., NH); 5.30–5.20 (*m*, 16 CH); 5.12 (*s*, PhCH_2O); 4.54–4.50 (*m*, H–C(3a)); 4.36–4.32 (*m*, H–C(6a)); 4.06 (*dd*, *J* = 18.15, 5.83, 1 H, CH_2NH); 3.94 (*dd*, *J* = 18.5, 5.19, 1 H, CH_2NH); 3.18–3.13 (*m*, H–C(4)); 2.93 (*dd*, *J*(6,6′) = 12.81, *J*(6,6a) = 5.03, H–C(6)); 2.74 (*d*, *J*(6,6′) = 12.79, H–C(6)); 2.71–2.41 (*m*, 16 CH_2); 2.31–2.27 (*m*, CH_2CO); 1.76–1.64 (*m*, 2 CH_2); 1.51–1.43 (*m*, CH_2); 1.29 (*d*, *J* = 6.33, Me); 1.28 (*d*, *J* = 6.09, 3 Me); 1.27 (*d*, *J* = 6.31, 7 Me); 1.27 (*d*, *J* = 5.85, 3 Me); 1.24 (*d*, *J* = 6.32, 2 Me). ^{13}C-NMR (100 MHz): 169.23; 169.20; 169.18; 169.12; 128.57; 128.32; 68.56; 67.70; 67.67; 67.66; 67.60; 66.45; 61.68; 60.14; 55.28; 41.23; 40.78; 40.70; 40.65; 40.46; 35.50; 28.12; 28.01; 25.41; 19.80; 19.75; 19.06. LSI-MS: 1791.0 (3, $[M+Na]^+$), 1769.2 (100, $[M+H]^+$), 1768.9 (92, M^+), 155.0 (35), 154.0 (16), 137.0 (13), 136.0 (15). Anal. calc. for $C_{83}H_{121}N_3O_{36}S$: C 56.36, H 6.89, N 2.38, S 1.81; found: C 56.32, H 6.90, N 2.46, S 1.90.

*(3*R*)-3-{{(3′*R*)-3′-{{2-{{5-[(3a*S*,4*S*,6a*R*)-Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}amino}acetyl}oxy}butanoyl}oxy}butanoic Acid* (**33**). Compound **31** (100 mg, 0.18 mmol) was hydrogenated according to *GP IIIa* in CF_3CH_2OH (10 ml). FC (CH_2Cl_2/MeOH 2:1) yielded **33** (144 mg, 82%). M.p. 141–144°. R_f 0.6 (CH_2Cl_2/MeOH 2:1). IR (KBr): 3279*w*, 2934*w*, 1738*s*, 1697*s*, 1455*w*, 1383*w*, 1302*w*, 1266*m*, 1177*w*, 1060*w*, 971*w*. ^{1}H-NMR (400 MHz, CD_3OD): 5.31–5.23 (*m*, 2 CH); 4.51–4.48 (*m*, H–C(3a)); 4.34–4.31 (*m*, H–C(6a)); 3.90 (*s*, CH_2NH); 3.24–3.19 (*m*, H–C(4)); 2.93 (*dd*, *J*(6,6′) = 12.74, *J*(6,6a) = 4.97, H–C(6)); 2.70 (*d*, *J*(6,6′) = 12.35, H′–C(6)); 2.61, 2.57 (*AB* of *ABX*, $J_{AB} = 15.65$, $J_{AX} = 7.78$, $J_{BX} = 5.53$); 2.58, 2.49 (*AB* of *ABX*, $J_{AB} = 15.33$, $J_{AX} = 7.81$, $J_{BX} = 5.41$); 2.30–2.26 (*m*, CH_2CO); 1.77–1.58 (*m*, 2 CH_2); 1.52–1.45 (*m*, CH_2); 1.29 (*d*, *J* = 6.40, Me); 1.27 (*d*, *J* = 6.38, Me). ^{13}C-NMR (100 MHz, CD_3OD): 176.57; 171.25; 170.74; 169.83; 169.81; 163.28; 61.65; 56.93; 42.27; 42.14; 41.79; 41.04; 36.40; 29.57; 29.41; 26.68; 20.08; 20.00. LSI-MS: 992.4 (3, $[M+Na]_2^+$), 991.4 (4, $[2M+2Na-H]^+$), 970.4 (2, $[2M+Na]^+$), 947.3 (2, $[2M+H]^+$), 497.0 (25, $[M+Na+H]^+$), 496.0 (100, $[M+Na]^+$), 495.0 (7, $[M+Na-H]^+$), 475.1 (18, $[M+H]^+$), 474.0 (60, M^+), 473.1 (5, $[M-H]^+$).

*α-{2-{{5-[(3a*S*,4*S*,6a*R*)-Hexahydro-2-oxo-1*H*-thieno[3,4-d]imidazol-4-yl]pentanoyl}amino}acetyl}-ω-hydroxyhexadecakis[(*R*)-oxy(1-methyl-3-oxopropane-1,3-diyl)]* (**34**). Compound **32** (300 mg, 0.17 mmol) was hydrogenated according to *GP IIIa* in CF_3CH_2OH (30 ml). FC (CH_2Cl_2/MeOH 8:1) yielded **34** (260 mg, 91%). White solid. M.p. 140–142°. R_f 0.7 (CH_2Cl_2/MeOH 8:1). IR (KBr): 3280*w*, 2931*w*, 1738*s*, 1698*s*, 1454*w*, 1383*w*, 1301*w*, 1267*m*, 1177*w*, 1060*w*, 970*w*. ^{1}H-NMR (400 MHz, $CD_3OD/CDCl_3$): 5.30–5.20 (*m*, 16 CH); 4.55–4.50 (*m*, H–C(3a)); 4.35–4.32 (*m*, H–C(6a)); 3.97 (*s*, CH_2NH); 3.18–3.11 (*m*, H–C(4)); 2.91 (*dd*, *J*(6,6′) = 12.79, *J*(6,6a) = 5.02, H–C(6)); 2.73 (*d*, *J*(6,6′) = 12.79, H′–C(6)); 2.72–2.40 (*m*, 16 CH_2); 2.31–2.26 (*m*, CH_2CO); 1.76–1.64 (*m*, 2 CH_2); 1.51–1.46 (*m*, CH_2); 1.29–1.23 (*m*, 16 Me). ^{13}C-NMR (100 MHz): 172.62; 128.58; 128.32; 68.56; 67.71; 67.64; 67.60; 66.44; 62.38; 60.09; 55.21; 41.24; 40.78; 40.68; 40.65; 40.46; 35.40; 28.12; 28.01; 26.41; 19.82; 19.76; 19.07. LSI-MS: 1718.0 (16, $[M+K]^+$), 1702.3 (41, $[M+Na+H]^+$), 1701.0 (46, $[M+Na]^+$), 1679.4 (100, $[M+H]^+$), 1650.1 (13), 1649.1 (12), 132.9 (14). Anal. calc. for $C_{76}H_{115}N_3O_{36}S$: C 54.40, H 6.85, N 2.51, S 1.91; found: C 54.28, H 6.87, N 2.62, S 1.98.

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