Synthesis of Oligo(3-hydroxybutanoate)(OHB)-Containing Peptides with High Binding Affinity to a Class-I-MHC Protein

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In the center of the immune system, there are *major histocompatibility* (MHC) protein/nonapeptide complexes which are recognized by T cells. The nonapeptides consist of three regions, an N-terminal one containing three amino-acid residues with a mandatory arginine in position 2, a C-terminal one with a lysine or arginine in position 9, and a central, variable one of five residues (*cf. Fig. 1*). We have now synthesized the first conjugates (**1**–**4**) of oligopeptides with oligo[(*R*)-3-hydroxybutanoates] (OHB) as analogs of MHC-binding peptides. Of the approaches chosen (*Scheme 1*), a fragment coupling of a hydroxy-butanoyl-amido ester (**17** and **19**) with an [(aminoalkanoyl)oxy]butanoyl chloride (**27**; *Scheme 3*), followed by two peptide-coupling steps (*Scheme 4*), turned out to be most efficient. The conjugates H-Gln-Arg-Leu-$(HB)_{3,4}$-Lys-OH (**1** and **2**) and H-Ala-Arg-Leu-$(HB)_{3,4}$-Lys-OH (**3** and **4**) were thus obtained in pure form. The conjugates **1** and **2** with N-terminal glutamine have a tendency to undergo cyclization with formation of a pyroglutamate residue (*cf. Fig. 2*). CD Measurements at different temperatures and so-called epitope-stabilization assays show that the complexes of the conjugates **2** and **4**, containing four HB units, with the HLA-B27 class-I-MHC protein are more stable than those of a model nonapeptide (C_{50} values of 2.25 and 1.60 μM *vs.* 10 μM), while the conjugates **1** and **3** with three HB units incorporated form less stable complexes (C_{50} values of 30 and 21 μM). The tetra(hydroxybutanoate)-peptide conjugates **2** and **4** are the first nonapeptide analogs for which the modification of the central part leads to increased affinities for a class-I-MHC protein, as compared to a model nonapeptide.

1. Introduction. – In learning and teaching, we are used to classify natural products and biopolymers according to their structures or to their biosynthetic origin (*Table*). However, there are, of course, also natural products in which components of different classes are covalently connected: glycoproteins, glycolipids, liposaccharides, lipoproteins, and, other, so-called *conjugates*[2])[3]). We have been investigating a group of biopolymers and biooligomers, consisting of (*R*)-3-hydroxybutanoate (HB) and other 3-hydroxyalkanoate residues, which have been known as microbial storage materials since 1926 [9], but which have yet to be generally recognized as the fifth class of ubiquitous biomacromolecules [1–3]. They have unambiguously been proved to be components of non-proteinaceous ion channels [10] [11] and to be minor components of

[1]) Part of the Ph.D thesis of *S.P.*, Dissertation No. 12570, ETH-Zürich, 1998.

[2]) Conjugates are not to be mixed up with noncovalent complexes between biomolecules. For recent papers on oligonucleotide conjugates, sapphyrin-lasalocid conjugates, and lipopeptides, see [6].

[3]) Conjugates of various biomolecules are sometimes also called *hybrids* [7] or (especially when nucleic acids are involved) *chimeric biomolecules* [8].

Table. *Biooligomers and Biopolymers, and Their Constituent Monomers, with the Corresponding Functions in Biochemistry and Biology.* The importance of poly[(*R*)-3-hydroxyalkanoates] as a fifth class of biopolymers, the most important representatives of which are poly[(*R*)-3-hydroxybutyrate] and poly[(*R*)-3-hydroxyvalerate] (PHB and PHV, resp.) and poly[(*S*)-malate] (*β*-PMA), has been outlined in several review articles [1–3]. The occurrence of natural conjugates between proteins and PHB/PHV has only recently been established [4] [5]; c- and s-PHB stand for complexing (mol. weight < 13000 Da or 150 HB units) and storage PHB (mol. weight *ca.* 10^6 Da or 12000 units).

Monomers metabolism	**Oligomers** regulation, recognition, signalling	**Polymers** catalysis, storage, transport, structure, information
amino acids	oligopeptides (*endorphines*)	polypeptides (*enzymes, silk*)
monosaccharides	oligosaccharides (*blood-group determinants*)	polysaccharides (*cellulose, starch*)
acetic acid, isoprene derivatives	isoprenoids (*steroids*)	polyisoprenoids (*rubber*)
nucleotides	oligonucleotides (*tRNA*)	polynucleotides (*DNA, RNA*)
hydroxyalkanoic acids	cP(3-HB) (*channel component, PHB-proteins*)	sP(3-HB) (*storage*)

proteins [4] [5] [12] in unknown structures[4])! We have now synthesized for the first time conjugates of oligopeptides with oligo(3-hydroxybutanoates) (OHB)[5]), reported herein, as well as conjugates of α- with β-oligopeptides[1])[6]). As targets of our syntheses, we chose the nonapeptides binding to the class-I major histocompatibility (MHC) proteins (and actually stabilizing them) which are in the center of the immune response [15]. These nonapeptides are known to have an N-terminal region of three amino acids, with a mandatory arginine in the 2-position, a C-terminal lysine, and a variable section of five amino acids in between (*Fig. 1,a*). Specifically, the six nonapeptides shown in *Fig. 1,b*, have been isolated from the arthritis-related[7]) class-I-MHC protein HLA-B27 [16]. The variable section of the nonapeptides comprises 15 bonds (*i.e.*, 3 atoms from each of the five amino acids), and it looks as if this part of the molecules, when bonded to the MHC protein, is more or less extended [17][8]).Thus, we decided to substitute the five central α-amino acids by three and by four β-hydroxy- or/and β-amino-acid residues (*Fig. 1,c*),

[4]) With the possible function of locking proteins into membranes.

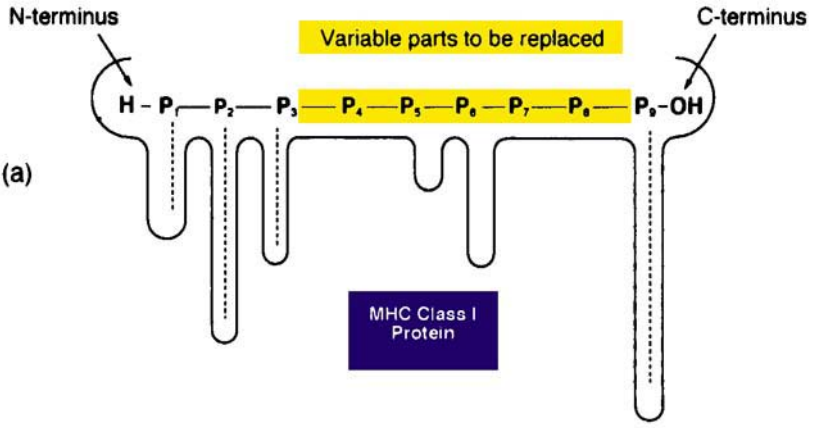
[5]) Peptides containing (usually single) 2-hydroxyalkanoic acid residues are well-known and are called depsipeptides when linear and peptolides when cyclic [13]. To the best of our knowledge, there is only one class of natural products containing 3-hydroxybutanoic acid and amino acids: the mycobactins, siderophores of mycobacteria. For a recent article on the synthesis of mycobactins with leading references, see [14a]. (*R*)-3-Hydroxybutanoic-acid-containing macrocyclic polylactones with nerve-growth-factor activity [14b] and with anti-fungal activity [14c] are also known.

[6]) The syntheses and bioassay of 'mixed' α-/β-peptides with class I-MHC-protein affinity will be published elsewhere.

[7]) Arthritis is believed to be an auto-immune disease!

[8]) Several X-ray crystal structures of nonapeptide-MHC complexes have been published and are used by one of our groups for extensive molecular modeling [18].

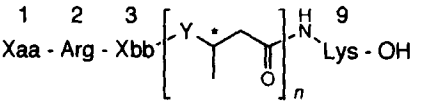
(a)



(b) **Amino acids series of some peptides eluted from HLA-B27**

P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	
H – Arg –	Arg	– Ile	– Lys	– Glu	– Ile	– Val	– Lys	Lys	– OH
H – Gln –	Arg	– Ile	– Asp	– Lys	– Pro	– Ile	– Leu	Lys	– OH
H – Arg –	Arg	– Ser	– Lys	– Glu	– Ile	– Thr	– Val	Arg	– OH
H – Arg –	Arg	– Tyr	– Gln	– Lys	– Ser	– Thr	– Glu	Leu	– OH
H – Ala –	Arg	– Leu	– Phe	– Gly	– Ile	– Arg	– Ala	Lys	– OH
H – Lys –	Arg	– Tyr	– Gln	– Lys	– Ser	– Thr	– Glu	Leu	– OH

(c)



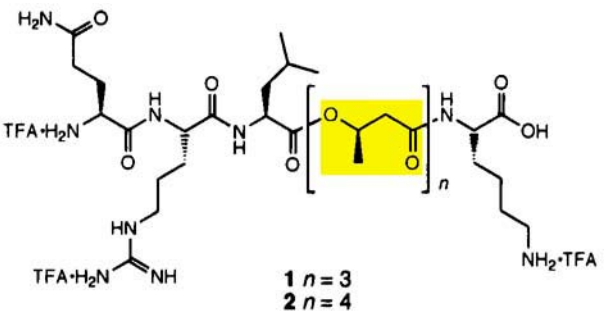
Xaa = Gln, Ala, Gly Y = O and/or NH

Xbb = Leu, Ala n = 3, 4

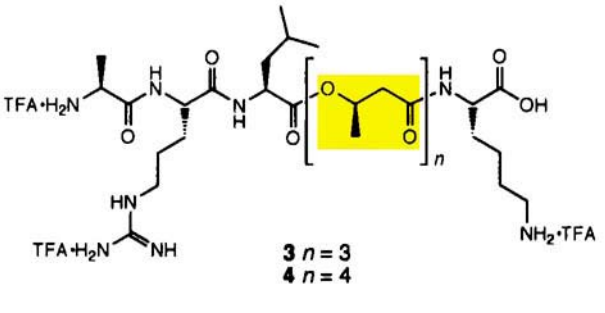
* (*R*) and/or (*S*) configuration

Fig. 1. *Complexes of a class-I-MHC protein with nonapeptides and nonapeptide analogs. a*) Binding pockets for the terminal regions of the nonapeptide (P_1-P_2-P_3 and P_9). *b*) Six natural nonapeptides which have been isolated from the class-I-MHC protein HLA-B27, with an arginine in position 2 and – with one exception – a positively charged side chain (Lys or Arg) in position 9; the great variability of the residues in positions 3 through 8 is obvious [15–18]. *c*) Nonapeptide analogs included in our investigations, with positions 4 to 8 occupied by 3-hydroxybutanoyl and 3-aminobutanoyl residues of various configurations. The synthesis of compounds **1**–**4** with tri- and tetra[(*R*)-3-hydroxybutanoates] $(HB)_3$ and $(HB)_4$ is described herein; for the β-amino-acid analogs, see *Footnote 6*.

creating nonapeptide analogs with a section, the extended structure of which[9]) would consist of a chain of 12 and 16 atoms, respectively. We describe here the syntheses of the conjugates **1**–**4** and mention results of affinity measurements with the HLA-B27 protein.



1 *n* = 3
2 *n* = 4



3 *n* = 3
4 *n* = 4

2. Synthetic Results. – The problems which could have been expected from the very beginning of this synthetic investigation are the following ones: The ester groups of the (OHB) section in the analogs **1**–**4** might not be stable under the acidic conditions of removal of protecting groups[10]) such as *tert*-butoxycarbonyl (Boc), *tert*-butyl esters (*ca.* 50% CF_3CO_2H (TFA) in CH_2Cl_2), or (pentamethylchroman)sulfonyl (Pmc) (*ca.* 80% TFA); under the basic conditions of (9*H*-fluoren-9-yl)methoxycarbonyl (Fmoc) deprotection (*sec*-amine in CH_2Cl_2), on the other hand, eliminative cleavages in the OHB segment ('crotonation') was to be expected[11]); finally free side-chain functional groups of the amino-acid residues could – intra- or intermolecularly – attack OHB ester groups with cleavage of the chain. Therefore, the methodology for the synthesis of the HB-containing peptide analogs **1**–**4** had to be carefully developed.

To have reference samples for current and for future investigations, we have first prepared some of the most simple conjugates between amino acids and HB, the *O*- and

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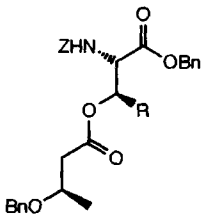
[9]) Oligo-(3-hydroxybutanoates) are known [1] [19] [20] to form 2_1 and 3_1 helices of *ca.* 6 Å pitch, and for β-peptides three helical secondary structures have been identified [21], so far. If present in their helical conformations, all of the segments (Y-CHMe-CH_2-CO)$_{3,4}$ (*Fig. 1, c*) would be too short to span the distance between the terminal binding domains for the nonapeptide.

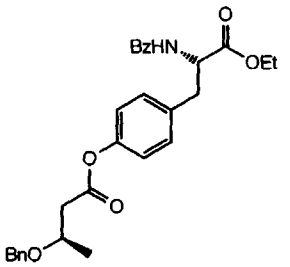
[10]) For protecting groups in general, see [22], in peptide synthesis, see [23].

[11]) The $(HB)_n$ unit contains β-(acyloxy)carbonyl functionalities, with a high tendency for elimination to α,β-unsaturated carbonyl (enoate) functionalities.

N-[3-(benzyloxy)butanoyl] derivatives **5**–**9**, from (benzyloxycarbonyl)-(Z)-serine, -threonine, and -lysine benzyl esters, from benzoyl(Bz)-tyrosine ethyl ester, and from *p*-toluenesulfonyl(Tos)-arginine methyl ester and (*R*)-3-(benzyloxy)butanoic acid. To make sure that we would also be able to identify elimination products, we also prepared the crotonoylated amino-acid derivatives **10**–**12** from Boc-serine, threonine or lysine *t*-Bu esters and crotonic acid.



5 R = H
6 R = Me



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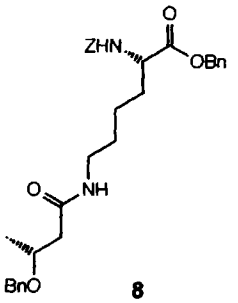

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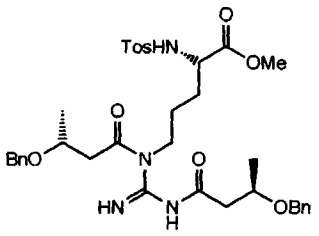

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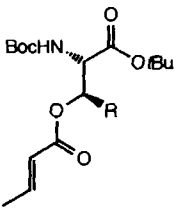
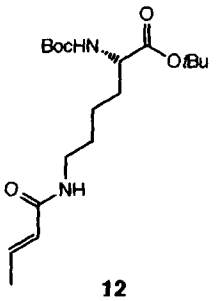


10 R = H
11 R = Me



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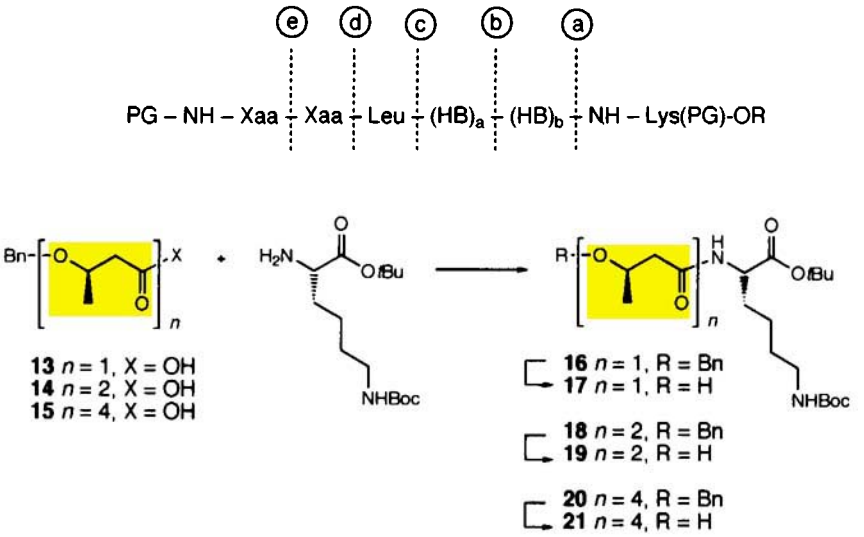
In view of the possible problems outlined above, we have not considered a totally linear synthetic route to the conjugates **1**–**4**. For a convergent, fragment-coupling approach, there were three alternatives to be considered, with respect to formation of bonds involving HB units (see **A** in *Scheme 1*): *a*) coupling by amide formation between HB and the C-terminal lysine, *b*) coupling by ester formation between two HB units, *c*) coupling by ester formation between Leu and a HB unit, eventually followed by *d*), *e*) peptide coupling between two amino-acid residues.

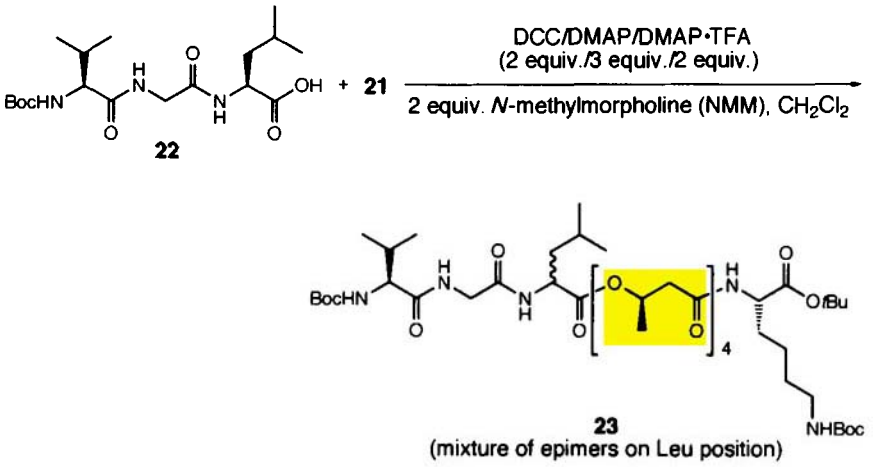
To find the best strategy, we first prepared Bn-(HB)$_n$-Lys(Boc)-O*t*Bu derivatives **16**, **18**, and **20** from H-Lys(Boc)-O*t*Bu (hydrochloride salt) and the *O*-Bn-protected mono-, di-, and tetra[(*R*)-3-hydroxybutanoates] **13**–**15** [19] by HOBt/EDC/Et_3N[12]) coupling

[12]) HOBt = 1-hydroxy-1*H*-benzotriazole; EDC = *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride. The coupling was carried out in CH_2Cl_2 at room temperature as is common in peptide synthesis. Coupling of the acid chloride derived from **15** [19], using various bases, with and without catalytic amounts of DMAP (4-(dimethylamino)pyridine), gave much poorer yields.

Scheme 1

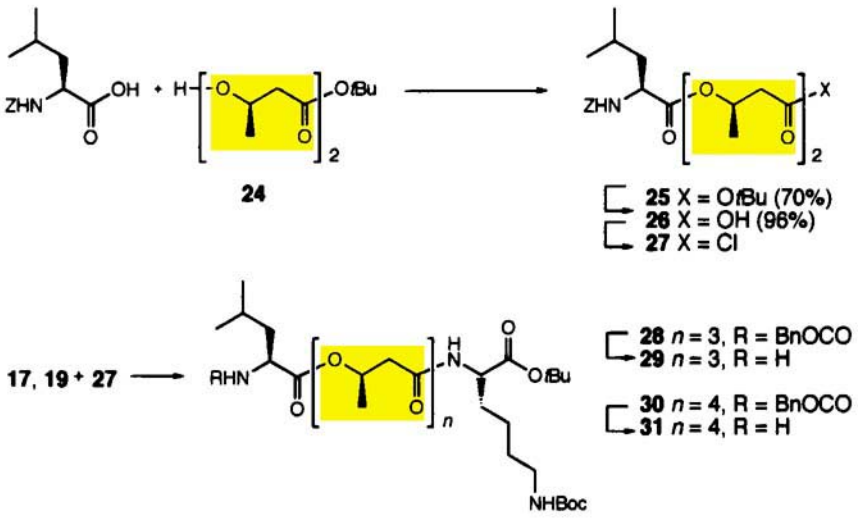


(70–87% yield, ⓐ in **A**). The benzyl ether could be readily cleaved by hydrogenolysis to give the hydroxy compounds **17**, **19**, and **21**. With the tetra-HB derivative **21**, we tested the possibility of coupling with a peptide (bond ⓒ in **A**): we chose the Boc-protected tripeptide **22**, available from a previous investigation [24], which was activated by a variety of methods; the best yield (30%) was obtained under the conditions [25] specified in *Scheme 2*; not surprisingly, the large amounts of base necessary to achieve this result led to appreciable epimerization at the Leu position so that only *ca.* 17% of a pure isomer **23** could be isolated.



Therefore, we next chose to try HB/HB coupling (ⓑ in **A**, and *Scheme 3*), and first esterified Z-Leu-OH with the *tert*-butyl di[(*R*)-3-hydroxybutanoate] **24** [19] (DCC/DMAP, CH_2Cl_2, room temperature, → **25**), cleaved the *t*-Bu ester (TFA/CH_2Cl_2 1:1 → **26**), activated the CO_2H group by conversion to the acid chloride **27**[13]) which was combined with the hydroxy compounds **17** or **19** (warming in CH_2Cl_2 from −75 to +20° in the presence of pyridine [26]). In both cases, the desired products **28** with three and **30** with four central HB units were formed in *ca.* 60% yield, after purification by flash chromatography (FC), in analytically pure form. Thus, this type of fragment coupling between two HB residues turned out to be superior to the one employing a tripeptide and a $(HB)_4$-Lys derivative (*cf. Scheme 1*, **A** ⓑ and ⓒ, and *Scheme 2*). Removal of the Z groups from **28** and **30** (Pd/C-H_2, MeOH) gave the corresponding compounds **29** and **31**, respectively, with free amino groups which were used for subsequent reactions without purification.

Scheme 3



With the conjugates **29** and **31**, we were ready to procede with the synthesis in a linear fashion, first attaching arginine, and then the N-terminal amino-acid residue (*Scheme 4*). For introducing arginine, we used the *N*-Fmoc-N^{ω}-Pmc-protected derivative (Pmc: 2,2,5,7,8-pentamethylchromane-6-sulfonyl), a commercially available building block, which was coupled[14]) with **29** and with **31**, using HOBt/EDC in CH_2Cl_2, to give the elongated, fully protected products **32** (50%) and **34** (61%), respectively. The N-terminus of both compounds was smoothly deprotected (5% piperidine in CH_2Cl_2 → **33**, **35**) and combined with HOBt/EDC-activated, trityl- and Boc-protected glutamine[15]), to give the nonapeptide analogs **36** and **37** with five acid-labile protecting groups.

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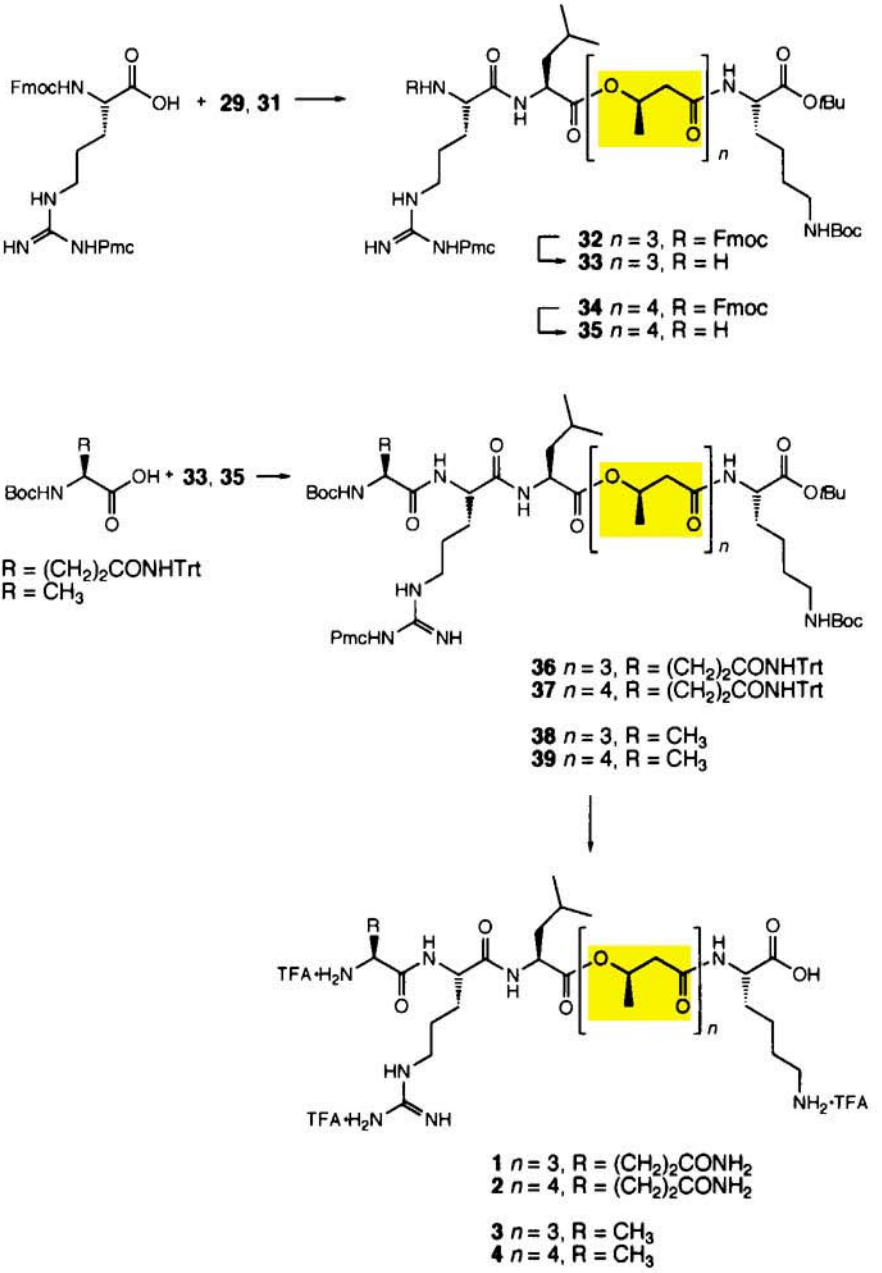
[13]) Compound **27** was generated by reaction of the acid with $(COCl)_2$ [26] (identified by NMR) and used directly for the coupling with **17** and **19**.

[14]) We also used the 'tri-Z-protected' arginine [27] which could be coupled with **31** in excellent yield (83%). However, the trick used by *Wünsch* and *Wendlberger* [28] (*i.e.*, removal of all three Z groups, protonation of the guanidino group (HBr), and direct coupling with the next amino acid) did not work in our case.

[15]) The commercial Boc-Gln(Trt)-OH was used as supplied.

Scheme 4


FmocHN
OH
+ 29, 31
RHN
HN
NHPmc
OtBu
NHBoc
32 n = 3, R = Fmoc
33 n = 3, R = H
34 n = 4, R = Fmoc
35 n = 4, R = H
BocHN
+ 33, 35
R = (CH2)2CONHTrt
R = CH3
PmcHN
36 n = 3, R = (CH2)2CONHTrt
37 n = 4, R = (CH2)2CONHTrt
38 n = 3, R = CH3
39 n = 4, R = CH3
TFA·H2N
NH2·TFA
1 n = 3, R = (CH2)2CONH2
2 n = 4, R = (CH2)2CONH2
3 n = 3, R = CH3
4 n = 4, R = CH3

One-step deprotection leading to the targets of our synthesis was achieved by treatment[16]) with 80% TFA in CH_2Cl_2. Unfortunately, the desired products **1** and **2** were contaminated with compounds which were formed by cleavage of HB ester bonds. While these impurities could be readily removed by preparative reversed-phase chromatography (80% H_2O, 20% MeCN, 0.1% TFA), there was also a tendency for the N-terminal glutamine to undergo cyclization to a pyroglutamate residue, a well-known phenomenon in peptide synthesis [30]. The NMR and MS detection of this by-product from compound **2** is shown in *Fig. 2*; the lactam formation took place even during the isolation procedure, on storage after HPLC purification, and in the course of ionization in the FAB mass spectrometer. Still, we were able to prepare very pure samples of the OHB-peptide conjugates **1** and **2**.

To circumvent the problem of pyroglutamate formation, and since it is known that position 1 of MHC-binding nonapeptides may be occupied by an alanine (see *Fig. 1, b*), we decided to synthesize the conjugates **3** and **4** with this simple amino acid at the N-terminus. Thus, arginine residues of the $(HB)_3$ and $(HB)_4$ derivatives **33** and **35** were coupled with Boc-Ala-OH under the usual conditions to give the fully protected analogs **38** and **39**, respectively (see *Scheme 4*). One-step removal of all the protecting groups afforded the desired products **3** and **4**, respectively; again there was appreciable loss of material by ester cleavages (according to analytical HPLC *ca.* 50%), but the compounds **3** and **4** could be readily isolated in pure form (as shown by NMR and mass spectrometry, after preparative HPLC), and they were stable under purification, isolation, and storage conditions.

3. Binding Affinity of the Conjugates 1–4 to an MHC Protein. – With the four analogs **1–4** available in 10–40-mg amounts, thermodynamic measurements and epitope-stabilization assays could be performed; the details are described by us in a separate paper [31]. The first test involves CD-spectrometric determination of the thermal stability ('*melting temperatures*') of the complex between the peptide analogs and the HLA-B27 protein (a class-I-MHC protein expressed by arthritis patients) [32]. The second test is an *in-vitro* cell assay, monitoring the stabilization of fluorescence-labeled HLA-B*2705-protein at the cell surface by the peptide analog, from which the molar affinity (C_{50} values)[17]) can be determined [33]. The 'melting-temperature' test yields values (as compared to model nonapeptides) of T_m = 63 and 62° (62°) for **3** and **4**, respectively; these compare with values of T_m = 46° (62°) for an ω-aminoundecanoic-acid analog [31] [32] of T_m = 70° (73°) for compounds with oligo(ethyleneglycol)ether loops [34], and of T_m = 56° (68°) for the complex with a phenanthridine derivative [35]. As can be seen from *Fig. 3*, the binding affinity[18]) of the two OHB-peptide conjugates with three HB units is clearly smaller than that of our reference nonapetide, while the affinity of the

[16]) Two equivalents of anisol have to be added to scavenge reactive intermediates formed upon removal of the Pmc group; the recommended [29] 50% TFA did not fully remove this protecting group in our case.

[17]) Molar concentration of the compound (here **1–4**) at 50% of maximum fluorescence (reference measurement at 10^{-4} M).

[18]) The samples of compounds **1** and **2** (with the N-terminal glutamine) were purified by HPLC directly before this test was performed, to make sure that no traces of pyroglutamate derivatives were present, which could have falsified the results of the test.

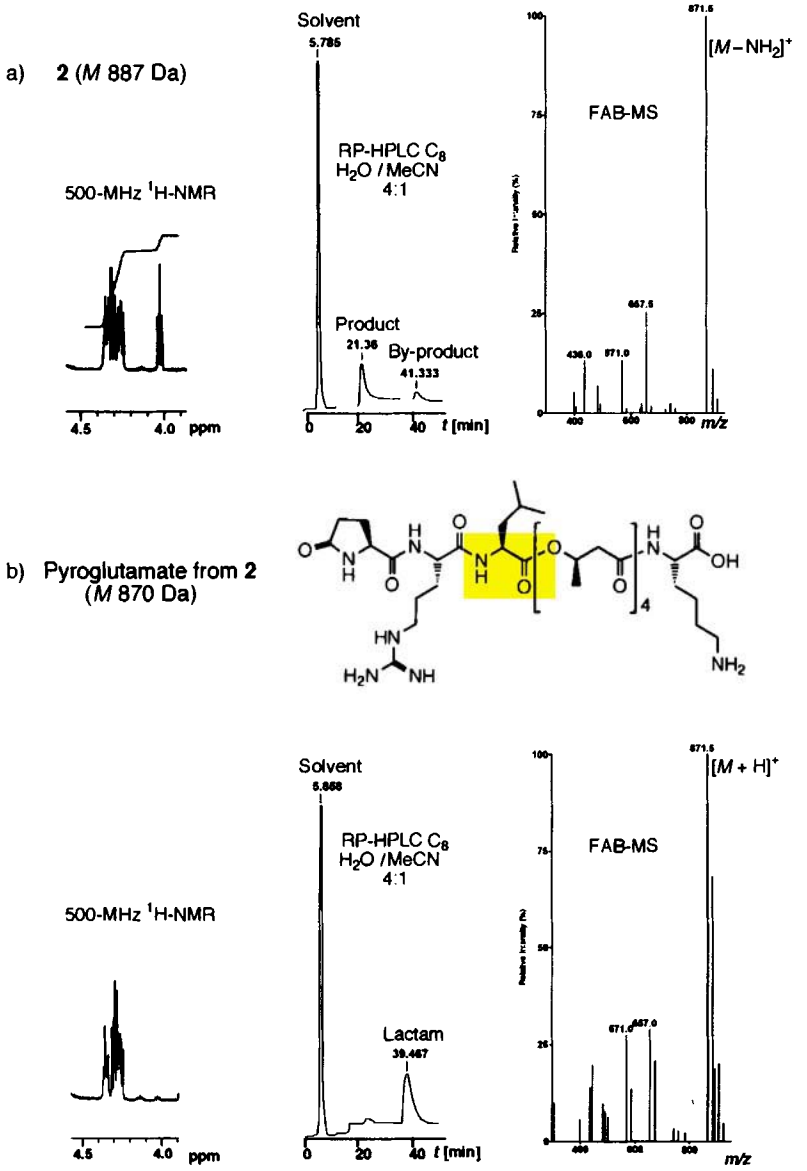


Fig. 2. *NMR, HPLC, and MS identification of the (HB)$_4$-peptide conjugate* **2** *and of the corresponding pyroglutamate cyclization product. a*) Characteristic NMR pattern of the NHC*H*(R) region with the typical 4-ppm signal from N-terminal glutamine with the lactam impurity indicated by a HPLC peak at much longer retention time, and FAB mass spectrum of **2**, with the highest-mass peak arising from $[MH - NH_3]^+$. *b*) Formula of the pyroglutamate, separated from the mixture with **2**, and NMR, HPLC, and MS traces.

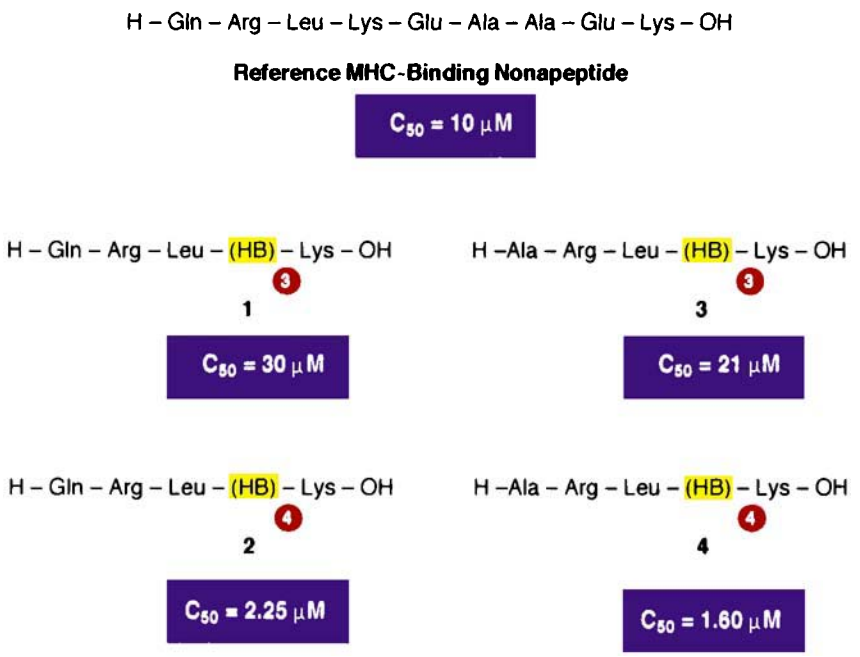


Fig. 3. *Affinities* (C_{50} values)[17]) *of a model nonapeptide and of the OHB-peptide conjugates* **1**–**4** *for the fluorescence-labeled class-I-MHC protein HLA-B*2705* [31] [33]. The values for the $(HB)_4$ derivatives are considered to indicate 'high affinity'. It appears that the $(HB)_3$ chain is too short for optimal binding of the two positively charged side chains in the terminal regions.

analogs with four HB units incorporated is *ca.* five times larger (values under 5 μM are considered to indicate high binding affinity).

Thus, we have discovered for the first time MHC-binding nonapeptide analogs, in which the five central amino acids are replaced by four (natural!) (*R*)-3-hydroxy butanoate residues, which exhibit higher affinities than the reference peptide! As will be shown[6]), the conjugates built of α- and β-peptides, which are chemically, and probably also biologically [21a] [36], more stable, have similar affinities in the epitope stabilization assay with the HLA-B*-2705 protein.

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Experimental Part

1. *General.* Abbreviations: DCC: dicyclohexylcarbodiimide, DMAP: 4-(dimethylamino)pyridine, EDC: *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide) hydrochloride, FC: flash chromatography, Fmoc: (9*H*-fluoren-9-yl)methoxycarbonyl, *GP*: *General Procedure*, HOBt: 1-hydroxy-1*H*-benzotriazole, h.v.: high vacuum, 0.01–0.1 Torr, NMM: *N*-methylmorpholin, Pmc: 2,2,5,7,8-pentamethylchromane-6-sulfonyl, TFA: trifluoroacetic acid, TDM: *N,N,N',N'*-tetramethyl-4,4'-diaminodiphenylmethane (= bis[4-(dimethylamino)phenyl]methane), (*R*)-3-HB: (*R*)-3-hydroxybutanoate. CH_2Cl_2 was stored over 4-Å molecular sieve, THF was freshly distilled over K

under Ar before use. Solvents for chromatography and workup procedures were distilled from *Sikkon* (anh. $CaSO_4$; *Fluka*). Et_3N was distilled from CaH_2 and stored over KOH. Isobutyl chloroformate was distilled and stored at + 4° under Ar. All indicated temp. were monitored with an internal thermometer (*Ebro TTX 690* digital thermometer). Amino-acid derivatives were purchased from *Bachem*, *Senn*, or *Alexxis*. All other reagents were used as received from *Fluka*. TLC: *Merck* silica gel *60* F_{254} plates; detection with UV and TDM. FC: *Fluka* silica gel *60* (40–63 mm); at *ca.* 0.2 bar. Anal. HPLC: *Knauer* HPLC system (pump type *64*, *EuroChrom 2000* integration package, degaser, UV detector (variable-wavelength monitor)). Prep. HPLC: *Knauer* HPLC system (pump type *64*, programmer *50*, UV detector (variable-wavelength monitor)). M.p.: *Büchi-510* apparatus; uncorrected. Optical rotations: *Perkin-Elmer-241* polarimeter (10-cm, 1-ml cell); at r.t. IR Spectra: *Perkin-Elmer-782* spectrophotometer. NMR Spectra: *Bruker AMX 400* (^{1}H, 400 MHz; ^{13}C, 100 MHz), *ARX 300* (^{1}H, 300 MHz; ^{13}C, 75 MHz) or *Varian Gemini 300* (^{1}H, 300 MHz; ^{13}C, 75 MHz); δ in ppm downfield from internal Me_4Si (= 0 ppm); J in Hz; some compounds show the presence of rotamers which are indicated. MS: *Hitachi Perkin-Elmer RHU-6M* (FAB) matrix 3-nitrobenzyl alcohol (3-NOBA). Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

2. *DCC/DMAP Ester Coupling: General Procedure 1 (GP 1)*. To a soln. of the hydroxy derivative (1 equiv.) in CH_2Cl_2 (0.1M), a soln. of the acid (1 equiv.) in CH_2Cl_2 (0.1M) was added under Ar, and the mixture was cooled to −5°. DCC (1.1 equiv.) and DMAP (0.1 equiv.) were added, and the resulting mixture was allowed to warm to r.t. and then stirred for 24 h. The mixture was diluted with Et_2O and washed with 1N HCl, sat. $NaHCO_3$, and sat. NaCl solns. The org. phase was dried ($MgSO_4$) and evaporated. FC and/or recrystallization afforded the pure product.

3. *HOBt/EDC Peptide Coupling: General Procedure 2 (GP 2)*. The free amine or the appropriate HCl salt (1 equiv.) was dissolved in CH_2Cl_2 (0.1M) under Ar and cooled to 0°. This was treated successively with Et_3N (1 or 3 equiv.), HOBt (1.25 equiv.), the acid (1 equiv.), and EDC (1.25 equiv.). The mixture was allowed to warm to r.t. and then stirred for 18 h. The mixture was diluted with CH_2Cl_2 and washed with 1N HCl, sat. $NaHCO_3$, and sat. NaCl solns. The org. layer was dried ($MgSO_4$) and evaporated. FC and/or recrystallization afforded the pure product.

4. *Removal of the Bn or Z Protecting Groups: General Procedure 3 (GP 3)*. Similarly to the procedure in [22], the Bn- or Z-protected compound was dissolved in MeOH (0.1M), and a catal. amount of 10% Pd/C was added. The apparatus was evacuated, flushed three times with H_2, and the mixture was stirred under an atmosphere of H_2 for *ca.* 8 h. Subsequent filtration through *Celite* and concentration under reduced pressure yielded the crude compound, which was identified by NMR and used without further purification.

5. *Cleavage of the Fmoc Protecting Group: General Procedure 4 (GP 4)*. The Fmoc-protected compound was dissolved in a 5–20% piperidine/CH_2Cl_2 soln. under Ar and cooled to 0°. The mixture was stirred 1 to 2 h, and concentration under reduced pressure yielded the crude amine, which was identified by NMR and used without further purification.

6. *Acid Chloride: General Procedure 5 (GP 5)*. At r.t. and under Ar, the acid (1 equiv.) was dissolved in CH_2Cl_2 (0.1M), and $(COCl)_2$ (1.5 equiv.) was added. The mixture was stirred 2 h at r.t., then a few drops of DMF were added, and the mixture was stirred for an additional 2 h, until gas evolution ceased. The solvent was removed and the resulting yellow oil was dried under h.v. and immediately used for the next reaction step.

7. *Final Deprotection: General Procedure 6 (GP 6)*. Under Ar and at 0°, the fully protected compound was dissolved in CH_2Cl_2/TFA (*v*/*v* 1:4), and anisole (2 equiv.) was added. The mixture was stirred 15 min to 1 h and then evaporated. The TFA salt was precipitated from Et_2O, dried under h.v., and purified by RP-HPLC.

8. *HPLC Analysis and Purification of the Conjugates* **1**–**4**. RP-HPLC Analysis was performed on a *Macherey-Nagel* C_8 column/*Nucleosil 100-5* C_8 (250 × 4 mm) by using a linear gradient of *A* (0.1% TFA in H_2O) and *B* (MeCN) at a flow rate of 1 ml/min with UV detection at 220 nm. Crude products were purified by prep. RP-HPLC on a *Macherey-Nagel* C_8 column/*Nucleosil 100-7* C_8 (250 × 21 mm) using gradient of *A* (0.1% TFA in H_2O) and *B* (MeCN) at a flow rate of 4 ml/min with UV detection at 214 nm and then lyophilized.

9. *Preparation of the Target Compounds* **1**–**4** *and of the Corresponding Intermediates. Z-Ser[(R)-3-HB-Bn]-OBn* (**5**). According to *GP 1*, the (*R*)-3-(benzyloxy)butanoic acid [19] (1.00 g, 5.1 mmol) was dissolved in CH_2Cl_2 (50 ml) and treated with Z-Ser-OBn (1.24 g, 4.0 mmol), DCC (1.44 g, 7.0 mmol), and DMAP (0.28 g, 2.3 mmol). Further purification by FC (pentane/Et_2O 11:9) gave **5** (2.00 g, 96%). Crystalline solid. M.p. 34–35°. $[\alpha]_D^{r.t.} = 0$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3432*w*, 3010*w*, 2974*w*, 1739*vs*, 1509*s*, 1455*w*, 1379*m*, 1340*w*, 1175*m*, 1084*m*, 1028*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.40–7.23 (*m*, 15 arom. H); 5.63 (br. *d*, J = 8.4, NH); 5.17 (*s*, OCH_2Ph); 5.11 (*s*, OCH_2Ph); 4.70–4.65 (*m*, CHO); 4.55–4.40 (*m*, CH_2O, OCH_2Ph); 3.94–3.87 (*m*, CHN); 2.53 ('*dd*', *ABX*, J = 7.1, 15.2, 1 H, CH_2CO); 2.36 ('*dd*', *ABX*, J = 5.6, 15.2, 1 H, CH_2CO); 1.21 (*d*, J = 6.2, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 171.15; 169.66; 156.09; 138.66; 135.28; 128.91; 128.86; 128.83; 128.65; 128.52; 128.42; 71.67;

70.88; 67.84; 67.35; 64.20; 53.58; 41.58; 19.74. FAB-MS: 1011 (10, $[2M + 1]^+$), 506 (100, $[M + 1]^+$). Anal. calc. for $C_{29}H_{31}NO_7$ (505.57): C 68.90, H 6.18, N 2.77; found: C 68.61, H 6.23, N 2.96.

*Z-Thr[(*R*)-3-HB-Bn]-OBn* (**6**). According to *GP 1*, the (*R*)-3-(benzyloxy)butanoic acid [19] (1.00 g, 5.1 mmol) was dissolved in CH_2Cl_2 (50 ml) and treated with Z-Thr-OBn (1.40 g, 4.0 mmol), DCC (1.44 g, 7.0 mmol), and DMAP (0.28 g, 2.3 mmol). Further purification by FC (pentane/Et_2O 2:1) gave **6** (1.82 g, 88%). Colorless oil. $[\alpha]_D^{r.t.} = +10.0$ ($c = 1.24$, $CHCl_3$). IR ($CHCl_3$): 3436*w*, 3010*w*, 1728*vs*, 1513*s*, 1455*w*, 1382*m*, 1314*w*, 1177*m*, 1066*m*, 1006*w*, 909*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.37–7.22 (*m*, 15 arom. H); 5.49–5.46 (*m*, CHO, NH); 5.14 (*s*, OCH_2Ph); 5.14–5.04 (*m*, OCH_2Ph); 4.56–4.44 (*m*, CHO, OCH_2Ph); 3.92–3.86 (*m*, CHN); 2.50 ('*dd*', *ABX*, $J = 6.8$, 14.9, 1 H, CH_2CO); 2.28 ('*dd*', *ABX*, $J = 5.9$, 14.9, 1 H, CH_2CO); 1.28 (*d*, $J = 6.5$, Me); 1.20 (*d*, $J = 6.2$, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 170.32; 170.05; 156.85; 138.63; 138.30; 136.30; 135.31; 128.86; 128.80; 128.61; 128.45; 71.74; 70.85; 70.70; 67.80; 67.61; 57.76; 41.82; 19.80; 19.75; 17.05. FAB-MS: 1039 (12, $[2M + 1]^+$), 520 (100, $[M + 1]^+$). Anal. calc. for $C_{30}H_{33}NO_7$ (519.60): C 69.35, H 6.40, N 2.70; found: C 69.50, H 6.38, N 2.47.

*Bz-Tyr[(*R*)-3-HB-Bn]-OEt* (**7**). According to *GP 1*, the (*R*)-3-(benzyloxy)butanoic acid [19] (310 mg, 1.6 mmol) was dissolved in CH_2Cl_2 (15 ml) and treated with Bz-Tyr-OEt (500 mg, 1.6 mmol), DCC (494 mg, 2.4 mmol), and DMAP (98 mg, 0.8 mmol). Further purification by recrystallization (pentane/Et_2O 1:1) gave **7** (470 mg, 60%). White solid. M.p. 90–91°. $[\alpha]_D^{r.t.} = +39.6$ ($c = 1.00$, $CHCl_3$). IR ($CHCl_3$): 3436*w*, 3005*w*, 1743*s*, 1656*s*, 1512*s*, 1382*m*, 1487*m*, 1309*w*, 1164*m*, 1092*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.76–7.73 (*m*, 2 arom. H); 7.51–7.44 (*m*, 3 arom. H); 7.43–7.26 (*m*, 5 arom. H); 7.15-7.12 (*m*, 2 arom. H); 6.98–6.95 (*m*, 2 arom. H); 5.65 (br. *d*, $J = 7.5$, NH); 5.07–5.02 (*m*, CHO); 4.63, 4.54 (*AB*, $J = 11.5$, OCH_2Ph); 4.21 (*q*, $J = 7.2$, CH_2); 4.17–4.10 (*m*, CHN); 3.30–3.20 (*m*, CH_2CHN); 2.86 ('*dd*', *ABX*, $J = 7.5$, 15.2, 1 H, CH_2CO); 2.67 ('*dd*', *ABX*, $J = 5.3$, 15.2, 1 H, CH_2CO); 1.34 (*d*, $J = 6.2$, Me); 1.27 (*t*, $J = 7.2$, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 171.84; 167.18; 150.05; 138.61; 134.12; 133.81; 132.09; 130.64; 128.92; 128.63; 127.99; 127.89; 127.27; 121.92; 72.17; 71.19; 61.88; 53.65; 42.23; 37.39; 19.85; 14.17. FAB-MS: 979 (16, $[2M + 1]^+$), 512 (15, $[M + Na]^+$), 490 (100, $[M + 1]^+$). Anal. calc. for $C_{29}H_{31}NO_6$ (489.57): C 71.15, H 6.38, N 2.86; found: C 71.03, H 6.36, N 2.83.

*Z-Lys[(*R*)-3-HB-Bn]-OBn* (**8**). According to *GP 2*, to a soln. of Z-Lys-OBn (1.32 g, 2.5 mmol) in CH_2Cl_2 (25 ml), Et_3N (1.05 ml, 7.5 mmol), HOBt (0.42 g, 3.1 mmol), a soln. of the (*R*)-3-(benzyloxy)butanoic acid [19] (0.5 g, 2.5 mmol) in CH_2Cl_2 (10 ml), and EDC (0.60 g, 3.1 mmol) were added. Further purification by recrystallization (Et_2O) gave **8** (1.04 g, 76%). White solid. M.p. 30–32°. $[\alpha]_D^{r.t.} = -21.0$ ($c = 0.93$, $CHCl_3$). IR ($CHCl_3$): 3434*w*, 3007*w*, 1718*vs*, 1654*s*, 1508*s*, 1455*w*, 1342*w*, 1177*w*, 1082*w*, 1044*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.35–7.25 (*m*, 15 arom. H); 6.22–6.16 (br. *m*, NH); 5.35 (br. *d*, $J = 8.1$, NH); 5.18–5.10 (*m*, OCH_2Ph); 5.10 (*s*, OCH_2Ph); 4.58 (*AB*, $J = 11.5$, 1 H, OCH_2Ph); 4.40 (*AB*, $J = 11.5$, 1 H, OCH_2Ph); 4.40–4.35 (*m*, CHO); 3.97–3.90 (*m*, CHN); 3.17–3.10 (*m*, CH_2N); 2.37 (*d*, $J = 5.9$, CH_2CHO); 1.90–1.70 (*m*, 1 H, CH_2); 1.70–1.60 (*m*, 3 H, CH_2); 1.45–1.35 (*m*, CH_2); 1.24 (*d*, $J = 5.9$, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.59; 171.45; 165.58; 156.31; 138.42; 135.56; 128.89; 128.79; 128.76; 128.58; 128.45; 128.37; 128.08; 128.02; 111.19; 72.74; 70.97; 67.29; 67.14; 53.83; 44.04; 38.86; 32.18; 28.99; 22.41; 19.58; 15.31. FAB-MS: 1093 (6, $[2M + 1]^+$), 547 (100, $[M + 1]^+$). Anal. calc. for $C_{32}H_{38}N_2O_6$ (546.67): C 70.31, H 7.01, N 5.12; found: C 70.27, H 6.88, N 5.03.

*Tos-Arg[(*R*)-3-HB-Bn]$_2$-OMe* (**9**). According to *GP 2*, to a soln. of Ts-Arg-OMe (500 mg, 1.3 mmol) in CH_2Cl_2 (8 ml), Et_3N (0.55 ml, 3.9 mmol), HOBt (221 mg, 1.6 mmol), a soln. of the (*R*)-3-(benzyloxy)butanoic acid [19] (256 mg, 1.3 mmol) in CH_2Cl_2 (4 ml) and EDC (314 mg, 1.6 mmol) were added. Further purification by FC (pentane/Et_2O 1:4) gave **9** (330 mg, 73%). Colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 13.16–13.10 (br. *m*, NH); 9.10–9.00 (br. *m*, NH); 7.72–7.68 (*m*, 2 arom. H); 7.32–7.24 (*m*, 12 arom. H); 5.39 (br. *d*, $J = 9.3$, NH); 4.61–4.48 (*m*, 2 OCH_2Ph); 4.09–3.92 (*m*, 3 H, CHO, CHN); 3.44 (*s*, Me); 3.44–3.38 (*m*, CH_2N); 2.80–2.39 (*m*, 2 CH_2CHO); 3.39 (*s*, Me); 1.79–1.57 (*m*, 2 CH_2); 1.24 (*m*, 2 Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 186.06; 173.80; 172.23; 155.94; 144.03; 139.34; 138.49; 136.84; 129.93; 128.60; 128.49; 127.92; 127.86; 127.53; 127.52; 72.95; 71.41; 70.89; 70.60; 55.48; 52.63; 48.78; 45.75; 39.98; 30.29; 25.03; 21.57; 20.16; 19.69. FAB-MS: 695 (100, $[M + 1]^+$). Anal. calc. for $C_{36}H_{46}N_4O_8S$ (694.85): C 62.23, H 6.67, N 8.06; found: C 61.91, H 6.43, N 7.83.

Boc-Ser(crotonoyl)-O^{t}Bu (**10**). According to *GP 1*, crotonic acid (200 mg, 2.3 mmol), dissolved in CH_2Cl_2 (25 ml) was treated with Boc-Ser-O^{t}Bu (607 mg, 2.3 mmol), DCC (719 mg, 3.5 mmol) and DMAP (136 mg, 1.1 mmol). Further purification by FC (pentane/Et_2O 4:1) gave **10** (403 mg, 53%). Crystalline solid. M.p. 90–91°. $[\alpha]_D^{r.t.} = +9.1$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3437*w*, 2978*w*, 2933*w*, 1717*vs*, 1497*s*, 1451*w*, 1369*m*, 1343*w*, 1153*s*, 1102*w*, 1030*w*, 969*w*, 846*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 6.97 ('*dq*', *ABX*, $J = 6.8$, 15.6, 1 H, $CHMe$); 5.81 ('*dq*', *ABX*, $J = 1.8$, 15.6, 1 H, CHCO); 5.30 (br. *d*, $J = 7.8$, NH); 4.54–4.44 (*m*, CH_2CHN); 4.36–4.26 (*m*, CHN); 1.87 (*dd*, $J = 1.8$, 6.8, Me); 1.44 (*s*, 2 *t*-Bu). ^{13}C-NMR (75 MHz, $CDCl_3$): 166.18; 146.07; 122.15;

82.83; 80.20; 64.73; 53.58; 28.35; 27.96; 18.09. FAB-MS: 659 (55, $[2M + 1]^+$), 330 (58, $[M + 1]^+$). Anal. calc. for $C_{16}H_{27}NO_6$ (329.40): C 58.34, H 8.26, N 4.25; found: C 58.50, H 8.50, N 4.23.

Boc-Thr(crotonoyl)-O^{t}Bu (**11**). According to *GP 1*, crotonic acid (159 mg, 1.8 mmol), dissolved in CH_2Cl_2 (20 ml), was treated with Boc-Thr-O^{t}Bu (514 mg, 1.8 mmol), DCC (572 mg, 2.8 mmol), and DMAP (111 mg, 0.9 mmol). Further purification by FC (pentane/Et_2O 17:2) gave **11** (370 mg, 60%). Crystalline solid. ^{1}H-NMR (300 MHz, $CDCl_3$): 6.95 ('*dq*', *ABX*, *J* = 6.8, 15.6, C*H*Me); 5.78 ('*dq*', *ABX*, *J* = 1.5, 15.6, CHCO); 5.50–5.38 (*m*, C*H*CHN); 5.20 (br. *d*, *J* = 9.3, NH); 4.38–4.30 (*m*, CHN); 1.86 (*dd*, *J* = 1.5, 6.8, Me); 1.46 (*s*, *t*-Bu); 1.40 (*s*, *t*-Bu). ^{13}C-NMR (75 MHz, $CDCl_3$): 145.73; 122.47; 82.62; 80.13; 70.94; 57.82; 28.35; 27.89; 18.04; 16.89. FAB-MS: 687 (19, $[2M + 1]^+$), 344 (46, $[M + 1]^+$). Anal. calc. for $C_{17}H_{29}NO_6$ (343.42): C 59.46, H 8.51, N 4.08; found: C 59.55, H 8.69, N 4.06.

Boc-Lys(crotonoyl)-O^{t}Bu (**12**). According to *GP 2*, to a soln. of Boc-Lys-O^{t}Bu (1 equiv., 3.0 mmol) in CH_2Cl_2 (30 ml), Et_3N (1.25 ml, 9.0 mmol), HOBt (0.51 g, 3.7 mmol), crotonic acid (0.26 g, 3.0 mmol), and EDC (0.72 g, 3.7 mmol) were added. Further purification by FC (pentane/Et_2O 1:2) gave **12** (940 mg, 85%). White solid. M.p. 79–80°. $[\alpha]_D^{r.t.} = -18.6$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3425*w*, 3005*w*, 1723*vs*, 1661*s*, 1512*s*, 1456*w*, 1348*w*, 1061*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 6.90–6.76 (*m*, C*H*Me); 5.82–5.75 (*m*, CHCO); 5.75–5.65 (*m*, NH); 5.20 (br. *d*, *J* = 12.1, NH); 4.20–4.10 (*m*, CHN); 3.38–3.24 (*m*, CH_2NH); 1.84 (*dd*, *J* = 1.5, 6.8, Me); 1.83–1.70 (*m*, 1 H, CH_2); 1.70–1.50 (*m*, 3 H, CH_2); 1.55–1.35 (*m*, CH_2); 1.45 (*s*, *t*-Bu); 1.43 (*s*, *t*-Bu). ^{13}C-NMR (75 MHz, $CDCl_3$): 125.35; 82.07; 53.71; 39.17; 32.86; 28.96; 28.40; 28.05; 22.55; 17.70. FAB-MS: 741 (44, $[2M + 1]^+$), 371 (86, $[M + 1]^+$). Anal. calc. for $C_{19}H_{34}N_2O_5$ (370.49): C 61.60, H 9.25, N 7.56; found: C 61.44, H 9.40, N 7.47.

*Bn-[(*R*)-3-HB]-Lys(Boc)-O^{t}Bu* (**16**). According to *GP 2*, to a soln. of the hydrochloric salt of H-Lys-(Boc)-O^{t}Bu (1.74 g, 5.1 mmol) in CH_2Cl_2 (40 ml), Et_3N (2.13 ml, 15.2 mmol), HOBt (0.86 g, 6.37 mmol), a soln. of the acid **13** [22] (1.00 g, 5.1 mmol) dissolved in CH_2Cl_2 (15 ml), and EDC (1.22 g, 6.37 mmol) were added. Further purification by FC (pentane/Et_2O 1:3) gave **16** (2.13 g, 87%). White foam. M.p. 44–45°. $[\alpha]_D^{r.t.} = -6.33$ (*c* = 1.18, $CHCl_3$). IR ($CHCl_3$): 3435*w*, 3364*w*, 2974*m*, 2864*w*, 1708*s*, 1662*s*, 1509*s*, 1455*w*, 1368*m*, 1160*s*, 1004*w*, 911*w*, 843*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.34–7.33 (*m*, 5 arom. H); 6.7 (br. *d*, *J* = 8.0, NH); 4.63 (*AB*, *J* = 11.5, 1 H, OCH_2Ph); 4.54–4.48 (br. *m*, NHBoc); 4.47 (*AB*, *J* = 11.5, 1 H, OCH_2Ph); 4.49–4.43 (*m*, CHN); 4,05–3.95 (*m*, C*H*OBn); 3.03–2.97 (*m*, CH_2NHBoc); 2.46 (*d*, *J* = 6, CH_2CO); 1.81–1.69 (*m*, 1 H, CH_2CHN); 1.64–1.49 (*m*, 1 H, CH_2CHN); 1.45 (*s*, *t*-Bu); 1.44 (*s*, *t*-Bu); 1.44–1.35 (*m*, CH_2); 1.29–1.17 (*m*, CH_2); 1.30 (*d*, *J* = 6.2, Me). ^{13}C-NMR (100 MHz, $CDCl_3$): 171.47; 170.63; 155.96; 138.26; 128.38; 81.86; 79.02; 72.41; 70.72; 52.38; 43.80; 40.20; 32.24; 29.48; 29.47; 28.42; 28.00; 22.34; 19.39. FAB-MS: 957 (9, $2M^+$), 479 (60, $[M + 1]^+$), 423 (18), 379 (23), 323 (100), 259 (16), 215 (16). Anal. calc. for $C_{26}H_{42}N_2O_6$ (478.63): C 62.25, H 8.84, N 5.85; found: C 65.24, H 8.78, N 5.88.

*HO-[(*R*)-3-HB]-Lys(Boc)-O^{t}Bu* (**17**). According to *GP 3*, **16** (1.89 g, 4.0 mmol) dissolved in MeOH (20 ml) was hydrogenated in presence of Pd/C (0.2 g) and of AcOH (0.1 ml). The hydroxy derivative **17** (1.50 g, 95%) was obtained as a colorless gel and used for the further steps without purification.

*Bn-[(*R*)-3-HB]$_2$-Lys(Boc)-O^{t}Bu* (**18**). According to *GP 2*, to a soln. of the hydrochloric salt of H-Lys-(Boc)-O^{t}Bu (5 g, 14.7 mmol) in CH_2Cl_2 (120 ml), Et_3N (6.14 ml, 44.1 mmol), HOBt (2.48 g, 18.4 mmol), a soln. of **14** [22] (4.13 g, 14.7 mmol) in CH_2Cl_2 (60 ml), and EDC (3.52 g, 18.4 mmol) were added. Further purification by FC (pentane/Et_2O 1:3) gave **18** (5.86 g, 71%). Colorless oil. $[\alpha]_D^{r.t.} = +4.96$ (*c* = 1.14, $CHCl_3$). IR ($CHCl_3$): 3446*w*, 2980*m*, 2934*w*, 1725*s*, 1682*s*, 1509*s*, 1455*w*, 1368*m*, 1161*m*, 1086*w*, 844*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.32–7.26 (*m*, 5 arom. H); 6.26 (br. *d*, *J* = 7.3, NH); 5.28–5.22 (*m*, CHO); 4.64–4.57 (br. *m*, NHBoc); 4.54, 4.48 (*AB*, *J* = 11.5, OCH_2Ph); 4.44–4.39 (*m*, CHN); 4.04–3.98 (*m*, C*H*OBn); 3.06–3.02 (*m*, CH_2NHBoc); 2.66–2.36 (*m*, 2 CH_2CO); 1.77–1.74 (*m*, 1 H, CH_2); 1.73–1.62 (*m*, 1 H, CH_2); 1.61–1.57 (*m*, CH_2); 1.45 (*s*, *t*-Bu); 1.43 (*s*, *t*-Bu); 1.38–1.36 (*m*, CH_2); 1.29 (*d*, *J* = 6.3, Me); 1.23 (*d*, *J* = 6.2, Me). ^{13}C-NMR (100 MHz, $CDCl_3$): 171.46; 170.59; 169.02; 155.98; 138.33; 128.28; 127.64; 127.53; 82.01; 79.00; 72.06; 70.84; 68.16; 52.41; 42.73; 42.16; 40.07; 39.97; 32.06; 29.51; 28.37; 27.94; 22.22; 19.76; 19.64. FAB-MS: 565 (22, $[M + 1]^+$), 465 (52), 409 (100), 323 (11), 301 (12), 259 (11), 215 (14). Anal. calc. for $C_{30}H_{48}N_2O_8$ (564.73): C 63.81, H 8.57, N 4.96; found: C 63.73, H 8.44, N 5.02.

*HO-[(*R*)-3-HB]$_2$-Lys(Boc)-O^{t}Bu* (**19**). According to *GP 3*, **18** (5.4 g, 9.6 mmol) dissolved in MeOH (80 ml) was hydrogenated in presence of Pd/C (0.5 g) and of AcOH (0.1 ml). Compound **19** (4.48 g, 98%) was obtained as a colorless gel and used for the further steps without purification. A small amount was purified by FC (pentane/Et_2O 1:4) for complete analysis. $[\alpha]_D^{r.t.} = +0.79$ (*c* = 1.02, $CHCl_3$). IR ($CHCl_3$): 3451*w*, 3415*w*, 2981*w*, 2933*w*, 1712*s*, 1671*m*, 1507*m*, 1456*w*, 1364*m*, 1164*s*, 1051*w*, 979*w*, 835*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 6.46 (br. *d*, *J* = 7.4, NH); 5.34–5.27 (*m*, CHO); 4.85–4.82 (br. *m*, NHBoc); 4.50–4.44 (*m*, CHN); 4.26–4.18 (*m*, C*H*OH); 3.58–3.55 (br. *s*, OH); 3.11–3.07 (*m*, CH_2NHBoc); 2.52–2.50 (*m*, CH_2CHOH); 2.44–2.42 (*m*,

CH_2CO); 1.84–1.76 (*m*, 1 H, CH_2); 1.68–1.59 (*m*, 1 H, CH_2); 1.53–1.40 (*m*, CH_2); 1.46 (*s*, *t*-Bu); 1.43 (*s*, *t*-Bu); 1.39–1.30 (*m*, CH_2); 1.33 (*d*, J = 6.4, Me); 1.24 (*d*, J = 6.3, Me). ^{13}C-NMR (100 MHz, $CDCl_3$): 171.82; 171.72; 169.28; 156.13; 82.16; 79.01; 77.20; 67.98; 64.76; 52.45; 43.87; 42.39; 40.00; 32.09; 29.55; 28.36; 27.93; 22.79; 22.30; 19.73. FAB-MS: 949 (4, $[2M + 1]^+$), 475 (48, $[M + 1]^+$), 375 (56), 319 (100), 259 (9), 233 (14), 215 (13). Anal. calc. for $C_{23}H_{42}N_2O_8$ (474.60): C 58.21, H 8.92, N 5.90; found: C 58.41, H 8.96, N 5.94.

*Bn-[(*R*)-3-HB]$_4$-Lys(Boc)-O^{t}Bu* (**20**). According to *GP 2*, to a soln. of the hydrochloric salt of H-Lys(Boc)-O^{t}Bu (1.7 g, 5.0 mmol) in CH_2Cl_2 (50 ml), Et_3N (2 ml, 15.0 mmol), HOBt (0.84 g, 6.2 mmol), a soln. of **15** [22] (2.25 g, 5.0 mmol) in CH_2Cl_2 (10 ml), and EDC (1.19 g, 6.2 mmol) were added. Further purification by FC (pentane/Et_2O 1:4) gave **20** (2.56 g, 70%). Colorless oil. $[\alpha]_D^{r.t.} = +5.27$ (c = 0.36, $CHCl_3$). IR (film): 3349*m*, 2973*m*, 2970*w*, 1732*s*, 1681*s*, 1527*m*, 1450*w*, 1363*m*, 1302*w*, 1256*w*, 1179*m*, 1056*w*, 973*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.31–7.24 (*m*, 5 arom. H); 6.35 (br. *d*, J = 7.5, NH); 5.27–5.19 (*m*, 3 CHO); 4.69–4.67 (br. *m*, NHBoc); 4.52, 4.47 (*AB*, J = 11.4, OCH_2Ph); 4.46–4.39 (*m*, CHN); 4.05–3.95 (*m*, *CH*OBn); 3.07–3.01 (*m*, CH_2NHBoc); 2.64–2.35 (*m*, 4 CH_2CO); 1.83–1.72 (*m*, 1 H, CH_2CHN); 1.69–1.57 (*m*, 1 H, CH_2CHN); 1.55–1.43 (*m*, CH_2); 1.44 (*s*, *t*-Bu); 1.41 (*s*, *t*-Bu); 1.36–1.31 (*m*, CH_2); 1.28–1.22 (*m*, 4 Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 171.51; 170.64; 169.28; 169.21; 168.99; 156.06; 138.49; 128.33; 127.61; 127.53; 82.08; 79.06; 71.95; 70.81; 68.61; 67.60; 67.40; 52.49; 42.62; 42.15; 40.91; 40.14; 32.14; 29.61; 28.44; 28.01; 22.32; 19.08. FAB-MS: 737 (8, $[M + 1]^+$), 637 (59), 581 (100), 473 (11), 387 (8), 301 (9), 259 (9), 215 (13). Anal. calc. for $C_{38}H_{60}N_2O_{12}$ (736.90): C 61.94, H 8.21, N 3.80; found: C 62.06, H 8.23, N 3.72.

*HO-[(*R*)-3-HB]$_4$-Lys(Boc)-O^{t}Bu* (**21**). According to *GP 3*, **20** (0.182 g, 0.246 mmol) dissolved in MeOH (5 ml) was hydrogenated in the presence of Pd/C (0.018 g) and AcOH (0.01 ml) during 12 h. Compound **21** (0.136 g, 84%) was obtained as a colorless gel and used for the further reaction without purification. A small amount was purified by FC (hexane/AcOEt 1:2) for anal. purposes. $[\alpha]_D^{r.t.} = -2.41$ (c = 1.08, $CHCl_3$). IR ($CHCl_3$): 3455*w*, 3425*w*, 2984*w*, 2933*w*, 1728*s*, 1616*m*, 1507*m*, 1456*w*, 1364*m*, 1297*w*, 1174*s*, 1056*w*, 969*w*, 908*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 6.44 (br. *d*, J = 7.8, NH); 5.35–5.21 (*m*, 3 CHO); 4.69–4.67 (br. *m*, NHBoc); 4.47–4.41 (*m*, CHN); 4.25–4.15 (*m*, *CH*OH); 3.27 (br. *s*, OH); 3.10–3.06 (*m*, CH_2NHBoc); 2.65–2.35 (*m*, 4 CH_2CO); 1.88–1.61 (*m*, 2 CH_2); 1.58–1.43 (*m*, CH_2); 1.45 (*s*, *t*-Bu); 1.43 (*s*, *t*-Bu); 1.31–1.21 (*m*, 4 Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.33; 172.00; 169.87; 169.89; 169.56; 169.38; 156.38; 82.29; 68.85; 67.88; 68.64; 64.58; 52.61; 43.55; 42.71; 41.01; 40.91; 40.25; 35.80; 32.21; 29.69; 28.49; 28.07; 22.68; 22.42; 19.95; 19.83. FAB-MS: 1294 (2, $[2M + 1]^+$), 647 (20, $[M + 1]^+$), 547 (71), 491 (100), 387 (7), 301 (7), 259 (5), 215 (9). Anal. calc. for $C_{31}H_{54}N_2O_{12}$ (646.78): C 57.57, H 8.42, N 4.33; found: C 57.29, H 8.14, N 4.35.

*Boc-Val-Gly-Leu-[(*R*)-3-HB]$_4$-Lys(Boc)-O^{t}Bu* (**23**). Under Ar, DCC (63 mg, 0.15 mmol), DMAP (56 mg, 0.45 mmol), and DMAP · TFA (72 mg, 0.30 mmol) were dissolved in CH_2Cl_2 (3 ml). The acid **22** [24] (59 mg, 0.15 mmol) was introduced into the mixture and stirred under reflux. A soln. of **21** (100 mg, 0.15 mmol), dissolved in CH_2Cl_2 (2 ml) containing NMM (33 µl, 0.30 mmol), was added in 4 h to the mixture and stirred under reflux during 24 h. Some Et_2O was added to the soln. before filtration over *Celite* of the urea formed during the reaction. The filtrate was washed with 1N HCl, sat. $NaHCO_3$, sat. NaCl solns. and dried ($MgSO_4$). Further purification by FC (hexane/AcOEt 1:4) gave **23** (24 mg, 17%). White foam. ^{1}H-NMR (300 MHz, $CDCl_3$): 6.99–6.95 (br. *m*, NH); 6.95–6.88 (br. *m*, NH); 6.65 (br. *d*, J = 7.0, NH); 5.29–5.22 (*m*, 4 CHO); 5.23–5.20 (br. *m*, NHBoc); 4.79–4.76 (br. *m*, NHBoc); 4.60–4.54 (*m*, CHN); 4.46–4.41 (*m*, CHN); 4.16–3.85 (*m*, CHN, CH_2N); 3.11–3.05 (*m*, CH_2NHBoc); 2.69–2.38 (*m*, 4 CH_2CO); 2.18–2.11 (*m*, Me_2CH); 1.81–1.71 (*m*, 1 H, CH_2CHN); 1.60–1.21 (*m*, 2 CH_2CHN, 2 Me_2CH, 2 CH_2); 1.46 (*s*, *t*-Bu); 1.44 (*s*, 2 *t*-Bu); 1.30–1.24 (*m*, 4 Me); 1.00–0.91 (*m*, 4 Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 171.99; 171.68; 169.30; 169.19; 169.08; 168.73; 138.09; 138.05; 96.46; 82.14; 77.58; 77.21; 68.63; 68.44; 67.71; 52.54; 51.18; 42.80; 42.55; 40.90; 40.80; 40.76; 40.15; 32.11; 29.62; 28.56; 28.43; 28.33; 28.23; 28.01; 27.41; 25.67; 24.78; 23.18; 22.87; 22.35; 21.63; 19.93; 19.78; 19.73; 19.34; 17.61. FAB-MS: 1016 (22, $[M + 1]^+$), 916 (76). Anal. calc. for $C_{49}H_{85}N_5O_{17}$ (1016.25): C 57.91, H 8.43, N 6.89; found: C 57.49, H 8.07, N 6.69.

*Z-Leu-[(*R*)-3-HB]$_2$-O^{t}Bu* (**25**). According to *GP 1*, Z-Leu-OH (7.00 g, 26.4 mmol) was dissolved in CH_2Cl_2 (60 ml) and treated with **24** [22] (6.40 g, 26.4 mmol), DCC (7.40 g, 36.4 mmol), and DMAP (1.46 g, 1.2 mmol). Further purification by FC (pentane/Et_2O 2:1) gave **25** (8.60 g, 70%). Colorless oil. $[\alpha]_D^{r.t.} = -15.43$ (c = 1.01, $CHCl_3$). IR ($CHCl_3$): 3434*w*, 2960*m*, 1726*vs*, 1511*m*, 1455*w*, 1369*m*, 1308*m*, 1164*m*, 1102*w*, 1053*m*, 976*w*, 840*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.36–7.30 (*m*, 5 arom. H); 5.34–5.19 (*m*, 2 CHO); 5.34–5.19 (*m*, NH); 5.12, 5.08 (*AB*, J = 12.2, OCH_2Ph); 4.38–4.30 (*m*, CHN); 2.64 ('*dd*', *ABX*, J = 7.1, 15.6, 1 H, CH_2CO); 2.54 ('*dd*', *ABX*, J = 7.7, 15.5, 1 H, CH_2CO); 2.49 ('*dd*', *ABX*, J = 6.3, 15.6, 1 H, CH_2CO); 2.40 ('*dd*', *ABX*, J = 5.5, 15.5, 1 H, CH_2CO); 1.75–1.52 (*m*, Me_2CHCH_2); 1.43 (*s*, *t*-Bu); 1.28 (*d*, J = 6.4, Me); 1.25 (*d*, J = 6.4, Me); 0.96–0.92 (*m*, Me_2C). ^{13}C-NMR (100 MHz, $CDCl_3$): 172.15; 169.400; 169.06; 155.93; 136.41; 128.12; 128.06; 80.96; 77.24; 68.39; 68.04; 66.88; 52.64; 41.94; 41.73; 40.82; 28.03; 24.73; 22.85; 21.85; 19.75; 19.58.

FAB-MS: 987 (< 1, $[2M + 1]^+$), 494 (8, $[M + 1]^+$), 438 (49), 394 (68), 220 (10), 176 (22). Anal. calc. for $C_{26}H_{39}NO_8$ (493.60): C 63.27, H 7.96, N 2.84; found: C 63.24, H 7.81, N 2.90.

Z-Leu-[(R)-3-HB]$_2$-OH (**26**). Under Ar, **25** (8.48 g, 17.2 mmol) was dissolved in CH_2Cl_2 (60 ml) and treated with TFA (40 ml). The reaction was completed after 12 h. Compound **26** (7.24 g, 96%) so obtained was used for the next step without further purification, only a small amount was purified by FC (pentane/Et_2O 1:3) for complete analysis. $[\alpha]_D^{r.t.} = -29.5$ ($c = 0.94$, $CHCl_3$). IR ($CHCl_3$): 3425*w*, 3312*w*, 2964*m*, 1728*vs*, 1712*vs*, 1523*w*, 1456*w*, 1420*w*, 1384*w*, 1307*m*, 1138*w*, 1107*w*, 1051*m*, 979*w*, 912*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, two rotamers): 9.5–9.0 (br. *m*, OH); 7.37–7.28 (*m*, 5 arom. H); 7.01 (br. *d*, $J = 8.7$, 0.33 H, NH); 5.46–5.39 (*m*, 2 CHO); 5.37–5.32 (*m*, 0.66 H, NH); 5.20–5.06 (*m*, OCH_2Ph); 4.46–4.40 (*m*, 0.66 H, CHN); 4.26–4.20 (*m*, 0.33 H, CHN); 2.65–2.44 (*m*, 2 CH_2CO); 1.73–1.43 (*m*, Me_2CHCH_2); 1.29–1.25 (*m*, 1.5 Me); 1.18–1.16 (*m*, 0.5 Me); 0.97–0.90 (*m*, 1.5 Me); 0.88–0.86 (*m*, 0.5 Me). ^{13}C-NMR (100 MHz, $CDCl_3$, two rotamers): 174.79; 172.74; 171.56; 169.53; 169.15; 158.06; 156.69; 135.82; 135.71; 128.47; 128.26; 128.19; 128.09; 127.98; 77.20; 69.32; 69.07; 68.32; 67.58; 67.44; 37.34; 53.24; 52.23; 41.69; 41.06; 40.94; 40.74; 40.64; 24.71; 24.47; 22.86; 22.80; 21.56; 21.24; 19.91; 19.81; 19.75; 19.26. FAB-MS: 875 (27, $[2M + 1]^+$), 438 (70, $[M + 1]^+$), 394 (100), 394 (68), 304 (15), 220 (7), 176 (11). Anal. calc. for $C_{22}H_{31}NO_8$ (437.49): C 60.40, H 7.14, N 3.20; found: C 60.31, H 7.09, N 3.19.

Z-Leu-[(R)-3-HB]$_3$-Lys(Boc)-O^{t}Bu (**28**). According to *GP 5*, the acid chloride **27** was prepared from **26** (1.80 g, 3.98 mmol) and $(COCl)_2$ (0.75 g, 5.97 mmol) in CH_2Cl_2 (10 ml). Then, **27** dissolved in CH_2Cl_2 (20 ml) was coupled with **17** (1.54 g, 3.98 mmol) at −78° in presence of pyridine (0.47 ml, 5.97 mmol). The mixture was allowed to warm to r.t. and stirred for 24 h. The mixture was diluted with CH_2Cl_2 and washed with 1N HCl, sat. $NaHCO_3$, sat. NaCl solns. and dried ($MgSO_4$). Further purification by FC (pentane/Et_2O, 1:3 to 1:4) yielded **28** (1.85 g, 58%). Transparent glassy compound. $[\alpha]_D^{r.t.} = -3.4$ ($c = 1.01$, $CHCl_3$). IR ($CHCl_3$): 3436*w*, 3374*w*, 2974*w*, 2933*w*, 2871*w*, 1728*s*, 1682*m*, 1512*m*, 1456*w*, 1369*m*, 1169*m*, 1046*w*, 979*w*, 907*w*, 841*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.35–7.28 (*m*, 5 arom. H); 6.57 (br. *d*, $J = 7.5$, NH); 5.64 (br. *d*, $J = 8.1$, NH); 5.30–5.21 (*m*, 3 CHO); 5.09 (*s*, OCH_2Ph); 4.69–4.67 (br. *m*, NHBoc); 4.49–4.42 (*m*, CHN); 4.39–4.33 (*m*, CHN); 3.08–3.06 (*m*, CH_2NHBoc); 2.69–2.33 (*m*, 3 CH_2CO); 1.82–1.47 (*m*, 3 CH_2, Me_2CH); 1.47 (*s*, *t*-Bu); 1.43 (*s*, *t*-Bu); 1.37–1.22 (*m*, CH_2); 1.29–1.24 (*m*, 3 Me); 0.95–0.91 (*m*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.08; 169.41; 128.74; 128.35; 128.26; 82.30; 68.86; 68.62; 67.86; 66.94; 52.70; 52.51; 42.46; 41.69; 40.91; 32.38; 29.65; 28.49; 28.03; 24.78; 22.92; 22.39; 21.85; 19.78; 19.70. FAB-MS: 830 (7, $[M + Na]^+$), 808 (19, $[M + 1]^+$), 708 (98), 652 (100). Anal. calc. for $C_{41}H_{65}N_3O_{13}$ (807.99): C 60.95, H 8.11, N 5.20; found: C 60.86, H 7.97, N 5.19.

H-Leu-[(R)-3-HB]$_3$-Lys(Boc)-O^{t}Bu (**29**). According to *GP 3*, **28** (0.87 g, 1.08 mmol) dissolved in MeOH (10 ml) was hydrogenated in presence of Pd/C (0.09 g) and of AcOH (0.1 ml). Compound **29** (0.72 g, quantitative) was obtained as a yellow oil and used for the further steps without purification.

Z-Leu-[(R)-3-HB]$_4$-Lys(Boc)-O^{t}Bu (**30**). According to *GP 5*, **27** was prepared from **25** (1.65 g, 3.75 mmol) and $(COCl)_2$ (0.708 g, 5.68 mmol) in CH_2Cl_2 (10 ml). Compound **27** dissolved in CH_2Cl_2 (20 ml) was then coupled with **19** (1.62 g, 3.42 mmol) at −78° in presence of pyridine (0.45 ml, 5.62 mmol). The mixture was allowed to warm to r.t. and stirred for 24 h. The mixture was then washed with 1N HCl, sat. $NaHCO_3$, sat. NaCl solns. and dried ($MgSO_4$). Further purification by FC (pentane/Et_2O 1:4) yielded **21** (1.83 g, 60%). $[\alpha]_D^{r.t.} = -3.45$ ($c = 1.00$, $CHCl_3$). IR ($CHCl_3$): 3444*w*, 2981*w*, 2861*w*, 1728*s*, 1682*m*, 1507*m*, 1456*w*, 1364*m*, 1297*w*, 1169*m*, 1056*m*, 974*w*, 902*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.37–7.31 (*m*, 5 arom. H); 6.37 (br. *d*, $J = 7.4$, NH); 5.46 (br. *d*, $J = 8.1$, NH); 5.30–5.22 (*m*, 4 CHO); 5.10 (*s*, OCH_2Ph); 4.67–4.64 (br. *m*, NHBoc); 4.48–4.43 (*m*, CHN); 4.37–4.32 (*m*, CHN); 3.09–3.07 (*m*, CH_2NHBoc); 2.68–2.37 (*m*, 4 CH_2CO); 1.84–1.47 (*m*, 3 CH_2, Me_2CH); 1.45 (*s*, *t*-Bu); 1.43 (*s*, *t*-Bu); 1.34–1.30 (*m*, CH_2); 1.30–1.25 (*m*, 4 Me); 0.99–0.92 (*m*, Me_2C). ^{13}C-NMR (100 MHz, $CDCl_3$): 172.35; 171.63; 169.31; 169.16; 169.14; 168.99; 156.02; 136.41; 128.47; 128.09; 128.03; 82.10; 79.04; 77.21; 68.56; 68.41; 67.65; 66.80; 52.60; 52.43; 42.58; 41.61; 40.81; 40.76; 40.70; 40.14; 32.19; 29.59; 28.41; 27.97; 24.71; 22.84; 22.31; 21.80; 19.77; 19.73; 19.60. FAB-MS: 894 (14, $[M + 1]^+$), 794 (100), 738 (93), 630 (5), 473 (4), 387 (5), 301 (6), 259 (4), 215 (8). Anal. calc. for $C_{45}H_{71}N_3O_{15}$ (894.08): C 60.45, H 8.00, N 4.70; found: C 59.78, H 7.94, N 4.59.

H-Leu-[(R)-3-HB]$_4$-Lys(Boc)-O^{t}Bu (**31**). According to *GP 3*, **30** (2.00 g, 2.22 mmol) dissolved in MeOH (20 ml) was hydrogenated in presence of Pd/C (0.20 g) and of AcOH (0.1 ml). **31** (1.34 g, 80%) was obtained as a yellow oil and used for the further steps without purification.

Fmoc-Arg(Pmc)-Leu-[(R)-3-HB]$_3$-Lys(Boc)-O^{t}Bu (**32**). According to *GP 2*, to a soln. of **29** (0.720 g, 1.08 mmol) in CH_2Cl_2 (10 ml), Et_3N (0.15 ml, 1.10 mmol), HOBt (0.182 g, 1.35 mmol), Fmoc-Arg(Pmc)-OH (0.826 g, 1.08 mmol), and EDC (0.258 g, 1.35 mmol) were added. Further purification by FC (hexane/AcOEt 1:2) gave **32** (0.710 g, 50%). White foam. $[\alpha]_D^{r.t.} = -1.8$ ($c = 1.00$, $CHCl_3$). IR ($CHCl_3$): 3344*w*, 2972*w*, 1734*s*, 1670*m*, 1551*m*, 1505*m*, 1444*w*, 1261*m*, 1101*m*, 1051*w*, 1015*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.76–7.74 (*m*, 2 arom. H); 7.60–7.57 (*m*, 2 arom. H); 7.41–7.36 (*m*, 2 arom. H); 7.31–7.28 (*m*, 2 arom. H); 7.19–7.10 (br. *m*, NH); 6.91–

6.90 (br. *m*, NH); 6.30–6.28 (br. *m*, 2 NH); 5.97–5.96 (br. *m*, NH); 5.89–5.88 (br. *m*, NH); 5.30–5.20 (*m*, 3 CHO); 4.82–4.80 (br. *m*, NHBoc); 4.57–4.50 (*m*, CHN); 4.40–4.27 (*m*, $CHCH_2O$); 4.40–4.27 (*m*, 2 CHN); 4.18 (*m*, $CHCH_2O$); 3.34–3.31 (*m*, 1 H, CH_2NHC); 3.21–3.19 (*m*, 1 H, CH_2NHC); 3.08–3.06 (*m*, CH_2NHBoc); 2.68–2.39 (*m*, 3 CH_2CO); 2.61 (*s*, Me); 2.59 (*s*, Me); 2.09 (*s*, Me); 1.88–1.49 (*m*, 6 CH_2, Me_2CH); 1.45–1.36 (*m*, 2 CH_2); 1.43 (*s*, *t*-Bu); 1.42 (*s*, *t*-Bu); 1.28–1.24 (*m*, 5 Me); 0.90–0.87 (*m*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.07; 169.90; 156.62; 144.14; 135.81; 135.36; 127.97; 127.32; 125.39; 124.13; 120.186; 82.57; 73.69; 68.77; 67.67; 53.90; 53.01; 51.20; 47.20; 40.73; 32.91; 29.70; 28.50; 28.05; 26.82; 24.82; 22.40; 21.59; 19.80; 18.62; 12.15. FAB-MS: 1318 (100, $[M + 1]^+$). Anal. calc. for $C_{68}H_{99}N_7O_{17}$ (1318.64): C 61.94, H 7.57, N 7.44; found: C 61.65, H 7.40, N 7.37.

*H-Arg(Pmc)-Leu-[(*R*)-3-HB]$_3$-Lys(Boc)-O^{t}Bu* (**33**). According to *GP 4*, **32** (200 mg, 0.15 mmol) was dissolved in CH_2Cl_2/piperidine (*v*/*v* 20:1). The cleavage was completed after 2 h. The solvent was removed *in vacuo*, and the yellow solid **33** was dried in h.v. and used without further purification.

*Fmoc-Arg(Pmc)-Leu-[(*R*)-3-HB]$_4$-Lys(Boc)-O^{t}Bu* (**34**). According to *GP 2*, to a soln. of **31** (0.945 g, 1.3 mmol) in CH_2Cl_2 (20 ml), Et_3N (0.18 ml, 1.3 mmol), HOBt (0.22 g, 1.62 mmol), a soln. of Fmoc-Arg(Pmc)-OH (0.861 g, 1.3 mmol) in CH_2Cl_2 (15 ml), and EDC (0.31 g, 1.62 mmol) were added. Further purification by FC (hexane/AcOEt 1:3) gave **34** (1.17 g, 61%). White foam. $[\alpha]_D^{r.t.} = -1.77$ ($c = 0.92$, $CHCl_3$). IR ($CHCl_3$): 3427*w*, 3354*w*, 2984*w*, 2943*w*, 2871*w*, 1723*s*, 1676*m*, 1620*w*, 1554*m*, 1507*m*, 1446*w*, 1374*w*, 1302*w*, 1164*m*, 1107*m*, 1051*w*, 1015*w*, 974*w*, 897*w*, 846*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.76–7.74 (*m*, 2 arom. H); 7.59–7.57 (*m*, 2 arom. H); 7.41–7.36 (*m*, 2 arom. H); 7.31–7.26 (*m*, 2 arom. H); 7.31–7.26 (*m*, NH); 6.65 (br. *d*, $J = 7.3$, NH); 6.28 (br. *m*, 2 NH); 5.85–5.82 (br. *m*, 2 NH); 5.30–5.21 (*m*, 4 CHO); 4.82–4.80 (br. *m*, NHBoc); 4.57–4.50 (*m*, CHN); 4.39–4.28 (*m*, $CHCH_2O$); 4.39–4.28 (*m*, 2 CHN); 4.18 (*t*, $J = 7.1$, $CHCH_2O$); 3.34–3.22 (*m*, CH_2NHC); 3.08–3.05 (*m*, CH_2NHBoc); 2.69–2.36 (*m*, 10 H, CH_2CO, CH_2); 2.60 (*s*, Me); 2.58 (*s*, Me); 2.09 (*s*, Me); 1.89–1.47 (*m*, 5 CH_2, Me_2CH); 1.45–1.41 (*m*, CH_2); 1.44 (*s*, *t*-Bu); 1.41 (*s*, *t*-Bu); 1.39–1.32 (*m*, CH_2); 1.28–1.19 (*m*, 6 Me); 0.89–0.85 (*m*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.15; 171.82, 171.50; 169.56; 169.46; 156.29; 143.90; 141.28; 135.57; 134.92; 133.72; 127.71; 125.15; 153.88; 119.96; 117.83; 82.25; 79.17; 77.74; 77.23; 76.16; 76.07; 73.55; 68.59; 67.69; 67.62; 67.04; 53.71; 52.79; 51.08; 47.14; 42.50; 42.38; 41.01; 40.91; 40.80; 40.57; 40.37; 40.26; 40.10; 32.85; 31.78; 30.65; 30.55; 30.43; 29.62; 28.68; 28.44; 28.00; 26.77; 25.02; 24.80; 22.85; 22.75; 22.40; 21.59; 21.43; 19.83; 19.68; 18.57; 17.52; 12.11. FAB-MS: 1404 (100, M^+). Anal. calc. for $C_{72}H_{105}N_7O_{19}S$ (1404.74): C 61.56, H 7.53, N 6.98; found: C 61.42, H 7.67, N 7.09.

*H-Arg(Pmc)-Leu-[(*R*)-3-HB]$_4$-Lys(Boc)-O^{t}Bu* (**35**). According to *GP 4*, **34** (1.1 g, 7.8 mmol) was dissolved in CH_2Cl_2/piperidine (*v*/*v* ratio 20:1). The cleavage was completed after 2 h. The solvent was removed *in vacuo*, and resulting **35** (yellow solid) was dried in h.v. and used without further purification. During an assay of purification (FC), the product decomposed in the column, and the pure product could not be isolated.

*Boc-Gln(Trt)-Arg(Pmc)-Leu-[(*R*)-3-HB]$_3$-Lys(Boc)-O^{t}Bu* (**36**). Both coupling strategies (HOBt/EDC and mixed anhydride) were tried for the synthesis of **36**: The yield of the reactions were very similar. The mixed-anhydride coupling method gave the best result and is described here. Under Ar and at $-10°$, amine **33** (1 equiv., 0.135 mmol) dissolved in THF (2 ml) was allowed to react with isobutyl chloroformate (18 mg, 0.135 mmol) and *N*-methylmorpholine (14 mg, 0.135 mmol). After 15 min stirring at $-10°$, the arginine Boc-Gln(Trt)-OH (66 mg, 0.135 mmol) was added to the mixture. The reaction was complete after 15-h stirring at r.t. The mixture was washed with 1N HCl, sat. $NaHCO_3$, and sat. NaCl solns. The org. layer was dried ($MgSO_4$), and the solvents were removed *in vacuo* to obtain the crude product. Further purification by FC (hexane/AcOEt 4:1) yielded **36** (140 mg, 66%). Transparent glass. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.27–7.17 (*m*, 15 arom. H, 2 NH); 7.11–7.02 (br. *m*, NH); 6.82 (br. *d*, $J \approx 6.8$, NH); 6.08–6.05 (br. *m*, 2 NH); 5.94–5.84 (br. *m*, NH); 5.80–5.70 (br. *m*, NH); 5.30–5.16 (*m*, 3 CHO); 4.88–4.80 (br. *m*, NHBoc); 4.50–4.30 (*m*, 3 CHN); 4.08–3.88 (*m*, CHN); 3.20–3.00 (*m*, CH_2NHC, CH_2NHBoc); 2.66–2.36 (*m*, 3 CH_2CO, $CH_2CONHTrt$); 2.56 (*s*, Me); 2.54 (*s*, Me); 2.09 (*s*, Me); 2.02–1.31 (*m*, 9 CH_2, Me_2CH); 1.43 (*s*, *t*-Bu); 1.42 (*s*, *t*-Bu); 1.39 (*s*, *t*-Bu); 1.29–1.22 (*m*, 5 Me); 0.85 (*d*, $J = 5.9$, 3 H, Me_2C); 0.84 (*d*, $J = 5.9$, 3 H, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 179.09; 172.39; 169.98; 169.72; 156.49; 153.71; 144.74; 135.76; 134.00; 128.92; 128.19; 127.27; 118.02; 82.38; 73.67; 70.75; 68.85; 67.80; 52.88; 51.07; 48.87; 42.47; 40.85; 40.17; 32.91; 29.65; 28.50; 28.38; 28.05; 26.81; 24.74; 22.84; 22.47; 21.66; 21.47; 19.75; 18.59; 17.54; 12.13. FAB-MS: 1567 (100, $[M + 1]^+$).

*Boc-Gln(Trt)-Arg(Pmc)-Leu-[(*R*)-3-HB]$_4$-Lys(Boc)-O^{t}Bu* (**37**). According to *GP 2*, to a soln. of **35** (1 equiv., 0.285 mmol) in CH_2Cl_2 (5 ml), Et_3N (0.04 ml, 0.285 mmol), HOBt (48 mg, 0.356 mmol), Boc-Gln(Trt)-OH (139 mg, 0.285 mmol), and then EDC (68 mg, 0.356 mmol) were added. Further purification by FC (hexane/AcOEt 3:1 → 4:1) gave **37** (270 mg, 61%). White foam. $[\alpha]_D^{r.t.} = -2.8$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3428*w*, 3353*w*, 2974*m*, 2933*w*, 2864*w*, 1733*s*, 1682*s*, 1548*m*, 1507*s*, 1451*w*, 1369*m*, 1164*s*, 1112*m*, 1056*w*, 907*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.38–7.30 (*m*, 2 NH); 7.26–7.16 (*m*, 15 arom. H, NH); 6.62 (br. *d*, $J = 7.4$, NH);

6.08–6.02 (br. *m*, 2 NH); 6.01–5.84 (br. *m*, NH); 5.90–5.82 (br. *m*, NH); 5.27–5.18 (*m*, 4 CHO); 4.85–4.79 (br. *m*, NHBoc); 4.47–4.33 (*m*, 3 CHN); 4.10–4.02 (*m*, CHN); 3.17–2.98 (*m*, CH_2NHC, CH_2NHBoc); 2.64–2.35 (*m*, 4 CH_2CO, CH_2CONHTrt); 2.54 (*s*, Me); 2.52 (*s*, Me); 2.07 (*s*, Me); 2.02–1.31 (*m*, 9 CH_2, Me_2CH); 1.43 (*s*, *t*-Bu); 1.41 (*s*, *t*-Bu); 1.38 (*s*, *t*-Bu); 1.28–1.16 (*m*, 6 Me); 0.83 (*d*, *J* = 5.3, 3 H, Me_2C); 0.82 (*d*, *J* = 5.6, 3 H, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.53; 172.34; 171.93; 171.72; 169.85; 169.70; 169.65; 156.48; 153.70; 144.74; 135.73; 135.05; 134.00; 128.92; 128.14; 127.20; 124.06; 118.01; 82.24; 80.11; 73.67; 70.71; 68.75; 68.44; 67.83; 65.95; 52.78; 51.09; 42.54; 40.96; 40.52; 32.92; 31.96; 29.63; 28.05; 28.37; 28.05; 26.82; 24.73; 22.82; 22.45; 21.69; 21.48; 19.83; 19.67; 18.58; 17.53; 15.28; 12.15. FAB-MS: 1652 (100, $[M + 1]^+$). Anal. calc. for $C_{86}H_{125}N_9O_{21}S$ (1653.06): C 62.49, H 7.62, N 7.63; found: C 62.29, H 7.92, N 7.70.

*Boc-Ala-Arg(Pmc)-Leu-[(*R*)-3-HB]$_3$-Lys(Boc)-O^{t}Bu* (**38**). According to *GP 2*, to a soln. of **33** (310 mg, 0.235 mmol) in CH_2Cl_2 (5 ml), Et_3N (0.033 ml, 0.235 mmol), HOBt (40 mg, 0.294 mmol), Boc-Ala-OH (44 mg, 0.235 mmol), and EDC (56 mg, 0.294 mmol) were added. Further purification by FC (hexane/AcOEt 4:1) gave **38** (210 mg, 70%). White foam. $[\alpha]_D^{r.t.} = -14.1$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3435*w*, 3343*w*, 2974*m*, 2933*w*, 2882*w*, 1723*s*, 1666*s*, 1615*w*, 1553*s*, 1502*m*, 1451*w*, 1379*m*, 1302*w*, 1159*s*, 1107*m*, 1056*w*, 1010*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.29–7.22 (*m*, NH); 7.18–7.13 (*m*, NH); 6.93–6.86 (*m*, NH); 6.32–6.26 (br. *m*, 3 NH); 6.02–5.94 (br. *m*, NH); 5.32–5.20 (*m*, 3 CHO); 4.92–4.84 (br. *m*, NHBoc); 4.60–4.36 (*m*, 3 CHN); 4.20–4.12 (*m*, CHN); 3.32–3.16 (*m*, CH_2NHC); 3.12–3.02 (*m*, CH_2NHBoc); 2.68–2.38 (*m*, 3 CH_2CO); 2.59 (*s*, Me); 2.57 (*s*, Me); 2.10 (*s*, Me); 1.94–1.36 (*m*, 8 CH_2, Me_2CH); 1.44 (*s*, *t*-Bu); 1.42 (*s*, *t*-Bu); 1.41 (*s*, *t*-Bu); 1.32 (*d*, *J* = 7.15, Me); 1.29–1.24 (*m*, 5 Me); 0.88–0.86 (*m*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.38; 169.87; 156.63; 135.80; 135.15; 124.15; 118.07; 82.46; 73.71; 68.86; 68.50; 67.77; 42.50; 40.86; 40.40; 32.92; 29.64; 28.49; 28.34; 28.05; 26.81; 24.75; 22.86; 22.47; 21.60; 19.79; 18.56; 17.54. FAB-MS: 1267 (100, M^+). Anal. calc. for $C_{61}H_{102}N_8O_{18}S$ (1267.60): C 57.80, H 8.11, N 8.84; found: C 57.80, H 8.08, N 8.98.

*Boc-Ala-Arg(Pmc)-Leu-[(*R*)-3-HB]$_4$-Lys(Boc)-O^{t}Bu* (**39**). According to *GP 2*, to a soln. of **35** (170 mg, 0.120 mmol) in CH_2Cl_2 (5 ml), Et_3N (0.017 ml, 0.120 mmol), HOBt (20 mg, 0.150 mmol), Boc-Ala-OH (23 mg, 0.12 mmol), and EDC (29 mg, 0.150 mmol) were added. Further purification by FC (hexane/AcOEt 4:1) gave **39** (116 mg, 72%). White foam. $[\alpha]_D^{r.t.} = -11.4$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3456*w*, 3374*w*, 2974*m*, 2943*w*, 2861*w*, 1733*s*, 1679*m*, 1615*w*, 1543*m*, 1502*m*, 1461*w*, 1379*m*, 1297*m*, 1164*s*, 1107*m*, 1061*w*, 1020*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.33–7.25 (*m*, NH); 7.14–7.06 (*m*, NH); 6.70–6.62 (*m*, NH); 6.32–6.22 (br. *m*, 3 NH); 6.01–5.92 (br. *m*, NH); 5.32–5.18 (*m*, 4 CHO); 4.96–4.86 (br. *m*, NHBoc); 4.60–4.36 (*m*, 3 CHN); 4.20–4.10 (*m*, CHN); 3.30–3.21 (*m*, CH_2NHC); 3.12–3.02 (*m*, CH_2NHBoc); 2.69–2.37 (*m*, 4 CH_2CO); 2.59 (*s*, Me); 2.57 (*s*, Me); 2.10 (*s*, Me); 1.82–1.30 (*m*, 8 CH_2, Me_2CH); 1.44 (*s*, *t*-Bu); 1.43 (*s*, *t*-Bu); 1.41 (*s*, *t*-Bu); 1.34 (*d*, *J* = 7.16, Me); 1.29–1.25 (*m*, 6 Me); 0.89 (*d*, *J* = 5.6, 3 H, Me_2C); 0.88 (*d*, *J* = 5.6, 3 H, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 171.97; 156.60; 135.13; 124.15; 118.07; 84.50; 82.38; 73.71; 68.74; 67.82; 42.56; 40.90; 32.91; 31.94; 29.59; 28.50; 28.34; 28.05; 26.81; 24.79; 22.86; 22.49; 21.63; 19.82; 18.59. FAB-MS: 1353 (100, M^+). Anal. calc. for $C_{65}H_{108}N_8O_{20}S$ (1353.69): C 57.67, H 8.04, N 8.28; found: C 57.68, H 7.96, N 8.41.

*H-Gln-Arg-Leu-[(*R*)-3-HB]$_3$-Lys-OH* (**1**). According to *GP 6*, **36** (*ca.* 50 mg, 0.032 mmol) was dissolved in CH_2Cl_2/TFA (1 ml, *v*/*v* 1:4), and anisole (6.9 µl, 0.064 mmol) was added. The cleavage was completed after 30 min. The solvents were removed *in vacuo*, and the yellow resulting oil was precipitated in Et_2O. The white TFA salt was triturated five times in Et_2O and then purified by RP-HPLC (10% *B*, t_R 20.5) to give **1** (7 mg, 28%). ^{1}H-NMR (300 MHz, D_2O): 5.30–5.16 (*m*, 3 CHO); 4.42–4.26 (*m*, 3 CHN); 4.09–4.03 (*m*, CHN); 3.24–3.17 (*m*, CH_2NHC); 3.01–2.93 (*m*, CH_2NH_2); 2.74–2.50 (*m*, 3 CH_2CO); 2.42–2.36 (*m*, CH_2CONH_2); 2.16–1.40 (*m*, 7 CH_2, Me_2CH); 1.26–1.21 (*m*, 3 Me); 0.92 (*d*, *J* = 5.9, 3 H, Me_2C); 0.86 (*d*, *J* = 5.6, 3 H, Me_2C). FAB-MS: 824 (28, $[M + Na]^+$), 802 (79, $[M + 1]^+$), 785 (100).

*H-Gln-Arg-Leu-[(*R*)-3-HB]$_4$-Lys-OH* (**2**). According to *GP 6*, **37** (*ca.* 40 mg, 0.025 mmol) was dissolved in CH_2Cl_2/TFA (1 ml, *v*/*v* 1:4), and anisole (5.4 µl, 0.05 mmol) was added. The cleavage was completed after 30 min. The solvents were removed *in vacuo*, and the resulting yellow oil was precipitated in Et_2O. The white TFA salt was triturated five times in Et_2O and then purified by RP-HPLC (10% *B*, t_R 21.3) to give **2** (10 mg, 30%). ^{1}H-NMR (300 MHz, D_2O): 5.27–5.21 (*m*, 4 CHO); 4.86–4.63 (*m*, 3 CHN); 4.10–4.06 (*m*, CHN); 3.25–3.21 (*m*, CH_2NHC); 3.01–2.97 (*m*, CH_2NH_2); 2.67–2.59 (*m*, 4 CH_2CO); 2.44–2.39 (*m*, CH_2CONH_2); 2.21–1.40 (*m*, 7 CH_2, Me_2CH); 1.26–1.24 (*m*, 4 Me); 0.92 (*d*, *J* = 5.6, 3 H, Me_2C); 0.86 (*d*, *J* = 6.1, 3 H, Me_2C). FAB-MS: 910 (16, $[M + Na]^+$), 888 (52, $[M + 1]^+$), 871 (100), 674 (40), 657 (68).

*H-Ala-Arg-Leu-[(*R*)-3-HB]$_3$-Lys-OH* (**3**). According to *GP 6*, **38** (*ca.* 170 mg, 0.135 mmol) was dissolved in CH_2Cl_2/TFA (2 ml, *v*/*v* 1:4), and anisole (29.3 µl, 0.27 mmol) was added. The cleavage was completed after 15 min. The solvents were removed *in vacuo*, and the resulting yellow oil was precipitated in Et_2O. The white TFA salt was triturated five times in Et_2O and then purified by RP-HPLC (15–40% *B*, 30 min, t_R 7.5) to give **3** (*ca.* 40 mg, 40%). ^{1}H-NMR (300 MHz, D_2O): 5.28–5.12 (*m*, 3 CHO); 4.48–4.32 (*m*, 3 CHN); 4.04 (*q*, *J* = 7.1,

CHN); 3.19–3.15 (*m*, CH_2NHC); 2.96–2.91 (*m*, CH_2NH); 2.70–2.52 (*m*, 4 CH_2CO); 1.86–1.62 (*m*, 5 CH_2, Me_2CH); 1.46 (*d*, *J* = 7.1, Me); 1.59–1.36 (*m*, CH_2); 1.23–1.18 (*m*, 3 Me); 0.87 (*d*, *J* = 5.9, 3 H, Me_2C); 0.81 (*d*, *J* = 5.9, 3 H, Me_2C). FAB-MS: 1491 (3, $[2M + 1]^+$), 745 (100, $[M + 1]^+$).

*H-Ala-Arg-Leu-[(*R*)-3-HB]$_4$-Lys-OH* (**4**). According to *GP 6*, **39** (*ca.* 25 mg, 0.017 mmol) was dissolved in CH_2Cl_2/TFA (1 ml, *v*/*v* 1:4), and anisole (3.7 µl, 0.034 mmol) was added. The cleavage was completed after 15 min. The solvents were removed *in vacuo*, and the resulting yellow oil was precipitated in Et_2O. The white TFA salt was triturated five times in Et_2O and then purified by RP-HPLC (15–40% *B*, 30 min, t_R 10.9) to give **4** (*ca.* 6 mg, 40%). ^{1}H-NMR (300 MHz, D_2O): 5.38–5.20 (*m*, 4 CHO); 4.48–4.32 (*m*, 3 CHN); 4.13 (*q*, *J* = 7.1, CHN); 3.32–3.24 (*m*, CH_2NHC); 3.08–2.98 (*m*, CH_2NH); 2.82–2.60 (*m*, 4 CH_2CO); 1.94–1.40 (*m*, 6 CH_2, Me_2CH); 1.55 (*d*, *J* = 7.1, Me); 1.33–1.28 (*m*, 4 Me); 0.89 (*d*, *J* = 5.8, 3 H, Me_2C); 0.88 (*d*, *J* = 5.8, 3 H, Me_2C). FAB-MS: 1662 (3, $[2M + 1]^+$), 831 (100, $[M + 1]^+$).

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