Auxiliary Agents for the Peroral Administration of Peptide and Protein Drugs: Synthesis and Evaluation of Novel Pepstatin Analogues

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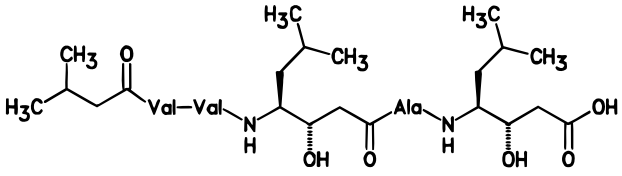
The peroral administration of (poly)peptide drugs requires the development of delivery systems, which provide a protective effect toward a gastrointestinal enzymatic attack. A promising strategy for such systems represents polymer−enzyme inhibitor conjugates in which the embedded therapeutic agent is protected. However, the practical use of polymer−inhibitor conjugates has so far been limited by high production costs of these auxiliary agents. To solve this problem for delivery systems shielding from pepsinic degradation, structurally simplified analogues of the pepsin inhibitor pepstatin A have been synthesized. The synthesis of tripeptide analogues, described by McConnell et al., led us to pursue further modifications varying the C-terminus. Our target to attach a spacer moiety—enabling the free access of pepsin to the inhibitor—should be combined with an attractive synthetic approach providing low production costs in large-scale preparation. Structure modifications comprised either the side chain of the third amino acid which served as starting compound designing the C-terminus (L-leucine, L-isoleucine, L-norvaline) as the length of the spacer link, simulated by a linear alkyl group (*n*-butyl, *n*-hexyl, and *n*-octyl). The inhibitory activities which have been evaluated by an enzyme assay were significantly dependent on the nature of the side chain, whereas the length of the spacer had no influence on the inhibitory effect. Analogues bearing the isobutyl or *n*-propyl moiety as side chain displayed a strong inhibitory effect which was comparable to that pepstatin A. These congeners represent promising auxiliary agents for the peroral administration of (poly)peptide drugs.

Introduction

For the efficient delivery of peptide and protein drugs by the peroral route, innovative and novel strategies are needed to overcome the enzymatic barrier of the gastrointestinal (GI) tract. Within these strategies, the combination of bioadhesive polymers with enzyme inhibitors has turned out to be a very promising approach.[1−3] Among such enzyme inhibitors, pepstatin A (**1**), a slow and tight-binding inhibitor of aspartyl proteinases,[4] has recently gained considerable interest as auxiliary agent for the peroral administration of epidermal growth factor (EGF). Itoh and Matsuo demonstrated in a double-blind controlled clinical study the enhanced healing of gastric ulcers after oral administration of EGF.[5] However, this effect is drastically reduced by the pepsinic degradation of this therapeutic polypeptide in the stomach.[6] To avoid the proteolysis of EGF after oral dosing, Bernkop-Schnürch and Dundalek have already generated a suitable bioadhesive drug delivery system for its peroral administration.[7] Thereby, pepstatin was covalently attached to a bioadhesive polymer in which the polypeptide drug was subsequently embedded. The bioadhesive polymer, on one hand, provides an intimate and prolonged contact with the gastric mucosa as well as a controlled and sustained drug release. On the other hand, the inhibitor guarantees a protective effect toward pepsinic degradation. To keep it concentrated on the delivery system and to avoid systemic toxic side effects caused by the inhibition of physiological essential, but pepstatin A-sensitive enzymes,[8−10] it was immobilized to the bioadhesive polymer sodium carboxymethylcellulose (→ **2**). The covalent attachment of pepstatin was thereby provided via a C_8-spacer supplying the free availability of the inhibitor for the enzyme.



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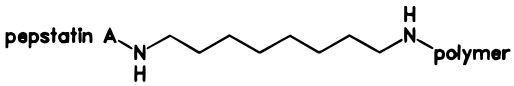

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However, although this system turned out to be very successful in various studies,[7] its practical use is strongly limited by the extensive costs of pepstatin. Therefore, it was the aim of this study to synthesize simplified pepstatin analogues bearing already an appropriate spacer moiety to provide the basis for linkage to suitable bioadhesive polymers. The development of easily accessible analogues should open the door to the

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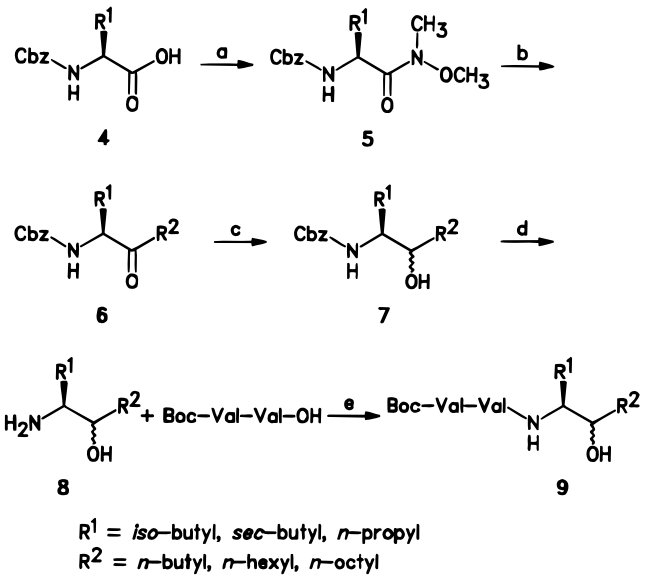
Scheme 1[a]

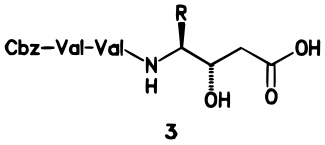


[a] Isobutyl chloroformate, *N*-methylmorpholine, *N*-methoxy-*N*-methylhydroxylamine hydrochloride, CH_2Cl_2, −15 °C to room temperature; (b) R^2MgBr, Et_2O, 0 °C; (c) $NaBH_4$, MeOH; (d) Pd/C/H_2, MeOH; (e) EEDQ, THF; R^1 = *n*-propyl, isobutyl, or *sec*-butyl; R^2 = *n*-butyl, *n*-hexyl, or *n*-octyl.

practical use of pepsin inhibitor−polymer conjugates as vehicles for the peroral administration of EGF as well as of further peptide and protein drugs which so far could not be administered by this route of application.

Chemistry

It was a clue to us that McConnell et al. have reported on tripeptide analogues **3** of pepstatin A, which can act as pepsin inhibitors.[11] In comparison with pepstatin A, these compounds contain the first three amino acid residues and therefore only one statine unit.



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R = *iso*–butyl, benzyl, *p*–OH–benzyl, *n*–butyl

The activity of these analogues depends on the side chain of the terminally located statine. When tested, eight analogues exhibited a comparably high inhibitory activity for compounds with a side chain derived from isoleucine—matching the structure of naturally occurring statine—which could even be exceeded by derivative **3** (R = C_4H_9) carrying a linear *n*-butyl side chain. The *N*-benzyloxycarbonyl group (instead of the isovaleryl moiety of pepstatin A) had no considerable influence on the inhibitory activity.

Regarding our target to immobilize the inhibitor on the matrix system, an additional modification either of the N-terminally located group or of the C-terminus became necessary. Although the C-terminus represents the sensible part of the molecule, we decided to perform a simplification of the statine unit, considering a simple and efficient synthetic approach. Our strategy is outlined in Scheme 1.

As starting compounds benzyloxycarbonyl (Cbz) protected amino acids (**4**) were prepared which were converted to the corresponding Weinreb amides (**5**) by reaction with *N*-methoxy-*N*-methylhydroxylamine hydrochloride after activation with isobutyl chloroformate.[12] The chosen amino acids comprised L-isoleucine (vide infra) (R^1 = *sec*-butyl), L-leucine (R^1 = isobutyl), and L-norvaline as amino acid with a linear side chain (R^1 = *n*-propyl) structurally comparable to the strongest inhibitor synthesized by McConnell et al.[11]

The following reductive alkylation was performed using Grignard reagents of different chain length [*n*-butylmagnesium bromide (R^2 = *n*-butyl), *n*-hexylmagnesium bromide (R^2 = *n*-hexyl), *n*-octylmagnesium bromide (R^2 = *n*-octyl)], affording the ketones **6** in good to excellent yields. Reduction with sodium borohydride resulted in a mixture of the diastereoisomeric amino alcohols **7** in a ratio between 1:2 and 1:4. Taking the steric hindrances caused by the side chain and the alkyl spacer into account, we suggest the predominant formation of the *4S*,*5S*-amino alcohol. Related data concerning the synthesis of pepstatin congeners and *vic*-amino alcohols with defined relative stereochemistry can be found in the literature.[13] The last two steps consisted in deprotection of the amino alcohol by catalytic hydrogenation to **8** and final coupling with Boc-Val-Val-OH, yielding the target peptides **9**.

Enzyme Assay

The enzyme inhibitory activity of all compounds (**3**−**9**) was determined using horseradish peroxidase as the pepsin sensitive protein which is in its native form able to oxidize *o*-phenylenediamine, leading to an orange-colored stain. Pepsin leads to a degradation of this enzyme. The remaining activity of peroxidase can therefore be set in correlation with the inhibition of pepsin effected by the tested compounds.[7]

Results and Discussion

Concerning a straight synthetic approach, we substitued the terminal statine unit of the simplified tripeptide analogues of pepstatin A, described by McConnell et al., by various amino alcohol residues. Whereas all compounds without the Boc-Val-Val moiety (**3**−**8**) did not show any inhibitory activity in the enzyme assay, all target analogues **9** displayed a strong inhibitory effect toward pepsin. Furthermore, we could demonstrate that the inhibitory activity is determined by the nature of the alkyl side chain on C-4, which is in good accordance with results obtained by McConnell et al.[11] The series derived from L-isoleucine as the third amino acid exhibited the significantly lowest inhibitory activities. In contrast, analogues with a linear side chain on C-4 (derived from L-norvaline) or with a isobutyl group on C-4 (derived from L-leucine) displayed a comparably stronger inhibitory activity. The variation of the spacer, represented by a linear alkyl group on C-5, had no significant influence on the inhibitory effect of all tested target compounds (**9**). Hence, the immobilization of these novel pepstatin analogues to bioadhesive polymers via spacers bound at C-5 should have no influence on their inhibitory potency. Results of inhibition studies are listed in Table 1. Pepstatin A derivatives, as described here, should therefore allow

Table 1. Inhibitory Effect of Pepstatin and Analogues toward the Pepsin Hydrolysis of Horseradish Peroxidase

compound	R^1	R^2	diastereoisomeric ratio 4*S*,5*S*:4*S*,5*R*	IC_{50} (M) ± SD ($n = 3$)
pepstatin A			1:0	$(5.9 \pm 2.5) \times 10^{-8}$
9a	*n*-propyl	*n*-butyl	2.5:1	$(9.1 \pm 3.2) \times 10^{-7}$
9b	*n*-propyl	*n*-hexyl	3:1	$(7.0 \pm 3.5) \times 10^{-7}$
9c	*n*-propyl	*n*-octyl	3:1	$(10.7 \pm 8.5) \times 10^{-7}$
9d	isobutyl	*n*-butyl	2:1	$(12.4 \pm 0.3) \times 10^{-7}$
9e	isobutyl	*n*-hexyl	2.5:1	$(8.3 \pm 3.6) \times 10^{-7}$
9f	isobutyl	*n*-octyl	2:1	$(7.9 \pm 2.7) \times 10^{-7}$
9g	*sec*-butyl	*n*-butyl	4.5:1	$(3.3 \pm 1.4) \times 10^{-5}$
9h	*sec*-butyl	*n*-hexyl	4:1	$(2.7 \pm 1.2) \times 10^{-5}$
9i	*sec*-butyl	*n*-octyl	4:1	$(1.5 \pm 0.9) \times 10^{-5}$

the production of very effective polymer−inhibitor conjugates for a reasonable price. Delivery systems based on such modified polymers are of high practical relevance, representing a useful tool for the peroral administration of mainly pepsinic degraded peptide and protein drugs.

In summary, compared to pepstatin A, analogues generated within this study display following advantages: (i) The presented inhibitors show a strong inhibitory activity, even by varying the spacer unit. (ii) The spacer moiety will allow an easy attachment of the inhibitor to the bioadhesive polymer. (iii) These derivatives can be easily produced under comparably low production costs.

Experimental Section

Melting points were determined on a Kofler hot plate apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer. ^1H and ^{13}C NMR spectra were measured on a Varian Unity Plus 300 spectrometer. ^1H spectra were referenced to tetramethylsilane (δ 0.0); in ^{13}C spectroscopy $CDCl_3$ served as the internal standard (δ 77.0). MS spectra were measured on Shimadzu QP 5000, Finnigan 8230, and Finnigan MAT 900S instruments. For inhibition studies a microtitration plate reader (Anthos Reader 2001) was used.

Flash chromatography was performed on MERCK silica gel 60, TLC on plastic sheets (MERCK silica gel 60 F_{254}). R_f values were determined using ethyl acetate−hexane (1:1) as the mobile phase. Tetrahydrofuran was dried over sodium/benzophenone; dichloromethane over phosphorus pentoxide.

***N*-(Benzyloxycarbonyl)-L-norvaline *N*-Methoxy-*N*-methyl amide (5α).** A solution of *N*-(benzyloxycarbonyl)-L-norvaline (61.75 mmol, 15.5 g) and *N*-methylmorpholine (123.5 mmol, 13.6 mL) in dry dichloromethane (150 mL), containing 2 g of molecular sieve (4 Å) was cooled to −15 °C prior to the slow addition of isobutyl chloroformate (8.0 mL). After 15 min *N*-methoxy-*N*-methylhydroxylamine hydrochloride (64 mmol, 6.22 g) was added. The reaction mixture was stirred for 1 h at −15 °C, and then the cooling bath was removed and the solution stirred for a further 3 h. Finally, the solution was washed with HCl (1 N, 2 × 20 mL), saturated aqueous $NaHCO_3$ solution (2 × 20 mL), and brine (2 × 20 mL). The organic layer was dried (Na_2SO_4) and evaporated, giving a yellowish oil which was purified by flash chromatography (ethyl acetate−hexane, 1:1) to afford 16.5 g (91%) of **5**α as a colorless oil: IR (KBr/liquid film, cm^{-1}) 1655 (CO−N(CH_3)-OCH_3), 1718 (N−CO−O); ^1H NMR ($CDCl_3$: δ 0.89 (br, 3H, CH_3), 1.36, 1.53, 1.65 (each m, 2H, 1H, 1H, CH_2CH_2), 3.15 (s, 3H, NCH_3), 3.73 (s, 3H, OCH_3), 4.72 (m, 1H, α-H), 5.05, 5.11 (AB system, 2H, $J = 12.0$ Hz, benzyl-H), 5.52 (br, 1H, NH), 7.30 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 13.4 (CH_3), 18.4, 34.5 (CH_2CH_2), 50.4 (NCH_3), 61.2 (OCH_3), 66.4 (benzyl-C), 127.6, 127.7, 128.2 (arom CH), 136.2 (arom C), 155.9 (N−CO−O); MS *m*/*z* 294.15 (M^+).

General Procedure for the Preparation of Ketones 6. To a stirred solution of amino acid *N*-methoxy-*N*-methylamide **5**α-γ (5 mmol) in dry tetrahydrofuran (20 mL) was added at 0 °C under an argon atmosphere the selected Grignard reagent (10 mmol of a ethereal solution). The mixture was stirred for 3 h (TLC control) and then quenched with HCl (1 N, 5 mL). The organic layer was separated, followed by extraction of the aqueous layer with diethyl ether (2 × 20 mL). The combined organic extracts were washed with brine, dried, evaporated, and purified by flash chromatography (ethyl acetate−hexane, 1:1) to afford **6a**−**i** as colorless oils or solids, respectively.

4-[(Benzyloxycarbonyl)amino]nonan-5-one (6a). This compound was synthesized from L-norvaline *N*-methoxy-*N*-methylamide (**5**α) and *n*-butylmagnesium bromide as the Grignard reagent: colorless solid; yield 74%; mp 40−42 °C; IR (KBr/liquid film, cm^{-1}) 3295 (NH), 1719 (N−CO−O), 1690 (C═O); ^1H NMR ($CDCl_3$) δ 0.83−0.95, 1.15−1.35, 1.36−1.57, 1.66−1.82 (each m, 6H, 4H, 3H, 1H, aliph H), 2.46 (m, 2H, 6-H), 4.37 (m, 1H, 4-H), 5.07 (s, 2H, benzyl-H), 5.50 (br d, 1H, $J = 6.6$ Hz, NH), 7.32 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 13.7, 13.8 (1-C, 9-C), 18.2, 22.2, 25.5, 33.8 (2-C, 3-C, 7-C, 8-C), 39.4 (6-C), 59.4 (4-C), 66.8 (benzyl-C), 128.0, 128.1, 128.4 (arom CH), 136.3 (arom C), 155.9 (N−CO−O), 209.2 (C═O); MS *m*/*z* 291.2 (M^+).

4-[(Benzyloxycarbonyl)amino]undecan-5-one (6b). This compound was synthesized from L-norvaline *N*-methoxy-*N*-methylamide (**5**α) and *n*-hexylmagnesium bromide as the Grignard reagent: colorless solid; yield 68%; mp 47−50 °C; IR (KBr/liquid film, cm^{-1}) 3295 (NH), 1718 (N−CO−O), 1690 (C═O); ^1H NMR ($CDCl_3$) δ 0.79−0.95, 1.17−1.60, 1.68−1.83 (each m, 6H, 11H, 1H, aliph. H), 2.42 (m, 2H, 6-H), 4.33 (m, 1H, 4-H), 5.03 (s, 2H, benzyl-H), 5.77 (br d, 1H, $J = 6.6$ Hz, NH), 7.26 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 13.5, 13.7 (1-C, 11-C), 18.1, 22.2, 23.1, 28.5, 31.2, 33.3 (2-C, 3-C, 7-C, 8-C, 9-C, 10-C), 39.3 (6-C), 59.2 (4-C), 66.4 (benzyl-C), 127.6, 127.7, 128.1 (arom CH), 136.2 (arom C), 155.8 (N−CO−O), 209.0 (C═O); MS *m*/*z* 319.2 (M^+).

4-[(Benzyloxycarbonyl)amino]tridecan-5-one (6c). This compound was synthesized from L-norvaline *N*-methoxy-*N*-methylamide (**5**α) and *n*-octylmagnesium bromide as the Grignard reagent: colorless solid; yield 76%; mp 55−58 °C; IR (KBr/liquid film, cm^{-1}) 3293 (NH), 1718 (N−CO−O), 1693 (C═O); ^1H NMR ($CDCl_3$) δ 0.85−0.95, 1.17−1.45, 1.45−1.70, 1.74−1.90 (each m, 6H, 12H, 3H, 1H, aliph H), 2.46 (m, 2H, 6-H), 4.38 (m, 1H, 4-H), 5.07 (s, 2H, benzyl-H), 5.56 (br d, 1H, $J = 6.6$ Hz, NH), 7.32 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) 13.5, 13.9 (1-C, 13-C), 18.3, 22.5, 23.5, 29.0, 29.3, 29.4, 31.7, 33.7 (2-C, 3-C, 7-C, 8-C, 9-C, 10-C, 11-C, 12-C), 39.7 (6-C), 59.4 (4-C), 66.8 (benzyl-C), 127.9, 128.0, 128.4 (arom CH), 136.3 (arom C), 155.9 (N−CO−O), 209.1 (C═O); MS *m*/*z* 348.2 ($M^+ + 1$).

4-[(Benzyloxycarbonyl)amino]-2-methylnonan-5-one (6d). This compound was synthesized from L-leucine *N*-methoxy-*N*-methylamide (**5**β) and *n*-butylmagnesium bromide as the Grignard reagent: colorless oil; yield 71%; IR (KBr/liquid film, cm^{-1}) 3335 (NH), 1721 (N−CO−O), 1702 (C═O); ^1H NMR ($CDCl_3$) δ 0.80−1.08, 1.25−1.43, 1.56, 1.70 (each m, 11H, 2H, 2H, 1H, aliph H), 2.48 (m, 2H, 6-H), 4.39 (m, 1H, 4-H), 5.08 (s, 2H, benzyl-H), 5.36 (br d, 1H, $J = 7.8$ Hz, NH), 7.33 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 13.7 (9-C), 21.7, 23.3, 24.8 (1-C, 2-C, 2-CH_3), 22.2, 25.6 (7-C, 8-C), 39.5, 40.8 (3-C, 6-C), 58.2 (4-C), 66.8 (benzyl-C), 127.9, 128.0, 128.4 (arom CH), 136.3 (arom C), 156.1 (N−CO−O), 209.7 (C═O); MS *m*/*z* 305.2 (M^+).

4-[(Benzyloxycarbonyl)amino]-2-methylundecan-5-one (6e). This compound was synthesized from L-leucine *N*-methoxy-*N*-methylamide (**5**β) and *n*-hexylmagnesium bromide as the Grignard reagent: colorless oil; yield 73%; IR (KBr/liquid film, cm^{-1}) 3330 (NH), 1718 (N−CO−O), 1705 (C═O); ^1H NMR ($CDCl_3$) δ 0.80−1.08, 1.20−1.43, 1.45−1.64, 1.70 (each m, 11H, 6H, 2H, 1H, aliph H), 2.48 (m, 2H, 6-H), 4.39 (m, 1H, 4-H), 5.07 (s, 2H, benzyl-H), 5.37 (br d, 1H, $J =$ 7.8, NH), 7.32 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 13.9 (11-C), 21.7, 23.3, 24.8 (1-C, 2-C, 2-CH_3), 22.4, 23.4, 28.8, 31.5 (7-C, 8-C, 9-C, 10-C), 39.8, 40.8 (3-C, 6-C), 58.2 (4-C), 66.8 (benzyl-C), 127.9, 128.0, 128.4 (arom CH), 136.3 (arom C), 156.1 (N−CO−O), 209.6 (C═O); MS *m*/*z* 333.5 (M^+).

4-[(Benzyloxycarbonyl)amino]-2-methyltridecan-5-one (6f). This compound was synthesized from L-leucine *N*-methoxy-*N*-methylamide (**5***β*) and *n*-octylmagnesium bromide as the Grignard reagent: colorless oil; yield 78%; IR (KBr/liquid film, cm^{-1}) 3436 (NH), 1711 (N−CO−O), 1699 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.82−1.05, 1.20−1.38, 1.48−1.65, 1.70 (each m, 11H, 10H, 2H, 1H, aliph H), 2.49 (t, 2H, J = 4.5 Hz, 6-H), 4.41 (m, 1H, 4-H), 5.08 (s, 2H, benzyl-H), 5.39 (br d, 1H, J = 7.5 Hz, NH), 7.33 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 13.9 (13-C), 21.7, 23.3, 24.8 (1-C, 2-C, 2-CH_3), 22.4, 23.4, 28.8, 29.6, 30.2, 31.9 (7-C, 8-C, 9-C, 10-C, 11-C, 12-C), 39.8, 40.8 (3-C, 6-C), 58.2 (4-C), 66.8 (benzyl-C), 127.9, 128.0, 128.4 (arom CH), 136.3 (arom C), 156.1 (N−CO−O), 209.8 (C═O); MS *m*/*z* 362.3 ($M^+ + 1$).

4-[(Benzyloxycarbonyl)amino]-3-methylnonan-5-one (6g). This compound was synthesized from L-isoleucine *N*-methoxy-*N*-methylamide (**5***γ*) and *n*-butylmagnesium bromide as the Grignard reagent: colorless solid; yield 75%; mp 40−43 °C; IR (KBr/liquid film, cm^{-1}) 3340 (NH), 1728 (N−CO−O), 1713 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.84−1.09, 1.29, 1.55, 1.90 (each m, 10H, 3H, 2H, 1H, aliph H), 2.47 (t, 2H, J = 7.2 Hz, 6-H), 4.34 (m, 1H, 4-H), 5.07 (s, 2H, benzyl-H), 5.46 (br d, 1H, J = 7.2 Hz, NH), 7.32 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 11.5, 13.7 (1-C, 9-C), 16.0 (3-CH_3), 22.1, 23.9, 25.4 (2-C, 7-C, 8-C), 37.0 (3-C), 40.7 (6-C), 64.2 (4-C), 66.8 (benzyl-C), 127.9, 128.0, 128.4 (arom CH), 136.2 (arom C), 156.3 (N−CO−O), 210.0 (C═O); MS *m*/*z* 306.2 ($M^+ + 1$).

4-(Benzyloxycarbonylamino)-3-methyl-undecan-5-one (6h). This compound was synthesized from L-isoleucine *N*-methoxy-*N*-methylamide (**5***γ*) and *n*-hexylmagnesium bromide as the Grignard reagent: colorless oil; yield 70%; IR (KBr/liquid film, cm^{-1}) 3340 (NH), 1721 (N−CO−O), 1710 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.83−1.15, 1.18−1.38, 1.57, 1.91 (each m, 10H, 7H, 2H, 1H, aliph. H), 2.47 (m, 2H, 6-H), 4.36 (m, 1H, 4-H), 5.09 (s, 2H, benzyl-H), 5.40 (br d, 1H, J = 7.8 Hz, NH), 7.34 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 11.6, 14.0 (1-C, 11-C), 16.2 (3-CH_3), 22.4, 23.4, 24.0, 28.8, 31.5 (2-C, 7-C, 8-C, 9-C, 10-C), 37.1 (3-C), 41.1 (6-C), 64.2 (4-C), 66.9 (benzyl-C), 128.0, 128.1, 128.5 (arom CH), 136.3 (arom C), 156.4 (N−CO−O), 210.0 (C═O); MS *m*/*z* 333.3 (M^+).

4-[(Benzyloxycarbonyl)amino]-3-methyltridecan-5-one (6i). This compound was synthesized from L-isoleucine *N*-methoxy-*N*-methylamide (**5***γ*) and *n*-octylmagnesium bromide as the Grignard reagent: colorless solid; yield 74%; mp 32−37 °C; IR (KBr/liquid film, cm^{-1}) 3305 (NH), 1715 (N−CO−O), 1687 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.82−1.05, 1.15−1.38, 1.55, 1.90 (each m, 10H, 11H, 2H, 1H, aliph H), 2.48 (t, 2H, J = 7.5 Hz, 6-H), 4.34 (m, 1H, 4-H), 5.08 (s, 2H, benzyl-H), 5.43 (br d, 1H, J = 8.4 Hz, NH), 7.33 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 11.6, 14.0 (1-C, 13-C), 16.1 (3-CH_3), 22.6, 23.4, 23.9, 28.8, 29.0, 29.6, 31.8 (2-C, 7-C, 8-C, 9-C, 10-C, 11-C, 12-C), 37.1 (3-C), 41.1 (6-C), 64.2 (4-C), 66.9 (benzyl-C), 128.0, 128.1, 128.4 (arom CH), 136.1 (arom C), 156.4 (N−CO−O), 209.4 (C═O); MS *m*/*z* 254.1 ($M^+ - 107$).

General Procedure for the Preparation of Alcohols 7. To a solution of ketone **6** (5 mmol) in methanol (25 mL) was added at room temperature 250 mg of sodium borohydride. After 30 min of stirring (TLC control), the solution was neutralized with HCl (2 N), concentrated, and partitioned between water (50 mL) and dichloromethane (20 mL). After extraction of the aqueous phase with dichloromethane (2 × 20 mL), the organic layers were combined, dried (Na_2SO_4), and brought to dryness, yielding alcohols **7** as white solids.

4-[(Benzyloxycarbonyl)amino]nonan-5-ol (7a): yield 85%; IR (KBr/liquid film, cm^{-1}) 3302 (OH), 1687 (N−CO−O); ^{1}H NMR ($CDCl_3$): δ 0.82−0.95, 1.20−1.55 (each m, 6H, 10H, aliph H), 2.16 (br, 1H, OH), 3.54−3.72 (m, 2H, 4-H, 5-H), 4.89 (br d, 1H, J = 7.8 Hz, NH), 5.09 (s, 2H, benzyl-H), 7.34 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.0 (1-C, 9-C), 19.4, 22.7 (2-C, 8-C), 27.9, 28.2, 31.2 (3-C, 6-C, 7-C), 54.6/55.6 (4-C), 66.8 (benzyl-C), 73.3/74.5 (5-C), 128.0, 128.1, 128.5 (arom CH), 136.5 (arom C), 156.8 (N−CO−O); MS *m*/*z* 294.3 ($M^+ + 1$).

4-[(Benzyloxycarbonyl)amino]undecan-5-ol (7b): yield 92%; IR (KBr/liquid film, cm^{-1}) 3314 (OH), 1686 (N−CO−O); ^{1}H NMR ($CDCl_3$) δ 0.85−0.97, 1.20−1.52 (each m, 6H, 14H, aliph H), 2.18 (br d, 1H, J = 4.8 Hz, OH), 3.65 (m, 2H, 4-H, 5-H), 4.89 (br d, 1H, J = 7.8 Hz, NH), 5.09 (s, 2H, benzyl-H), 7.34 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.0 (1-C, 11-C), 19.4, 22.6, 26.0, 29.3, 31.2, 31.7, 33.1 (2-C, 3-C, 6-C, 7-C, 8-C, 9-C, 10-C), 55.6 (4-C), 66.8 (benzyl-C), 74.6 (5-C), 128.0, 128.1, 128.5 (arom CH), 136.5 (arom C), 156.8 (N−CO−O); MS *m*/*z* 322.2 ($M^+ + 1$).

4-[(Benzyloxycarbonyl)amino]tridecan-5-ol (7c): yield 90%; IR (KBr/liquid film, cm^{-1}) 3317 (OH), 1686 (N−CO−O); ^{1}H NMR ($CDCl_3$) δ 0.83−0.95, 1.20−1.50 (each m, 6H, 18H, aliph H), 2.24 (br d, 1H, OH), 3.63 (m, 2H, 4-H, 5-H), 4.92 (br d, 1H, J = 7.8 Hz, NH), 5.09 (s, 2H, benzyl-H), 7.33 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.0 (1-C, 13-C), 19.4, 22.6, 26.0, 29.2, 29.5, 29.6, 31.2, 31.8, 33.1 (2-C, 3-C, 6-C, 7-C, 8-C, 9-C, 10-C, 11-C, 12-C), 55.6 (4-C), 66.8 (benzyl-C), 74.6 (5-C), 127.9, 128.0, 128.5 (arom CH), 136.5 (arom C), 156.8 (N−CO−O); MS *m*/*z* 350.2 ($M^+ + 1$).

4-[(Benzyloxycarbonyl)amino]-2-methylnonan-5-ol (7d): yield 91%; IR (KBr/liquid film, cm^{-1}) 3336 (OH), 1687 (N−CO−O); ^{1}H NMR ($CDCl_3$) δ 0.82−0.98, 1.18−1.56, 1.58−1.72 (each m, 9H, 8H, 1H, aliph H), 2.18 (br, 1H, OH), 3.50−3.80 (m, 2H, 4-H, 5-H), 4.86 (br d, 1H, J = 7.2 Hz, NH), 5.09 (s, 2H, benzyl-H), 7.33 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 14.0 (9-C), 21.6, 23.7, 24.7 (1-C, 2-C, 2-CH_3), 22.7, 28.2, 32.7, 38.0 (3-C, 6-C, 7-C, 8-C), 53.9 (4-C), 66.8 (benzyl-C), 74.8 (5-C), 128.0, 128.1, 128.5 (arom CH), 136.4 (arom C), 156.7 (N−CO−O); MS *m*/*z* 308.2 ($M^+ + 1$).

4-[(Benzyloxycarbonyl)amino]-2-methylundecan-5-ol (7e): yield 93%; IR (KBr/liquid film, cm^{-1}) 3334 (OH), 1687 (N−CO−O); ^{1}H NMR ($CDCl_3$) δ 0.82−0.98, 1.15−1.54, 1.56−1.70 (each m, 9H, 12H, 1H, aliph H), 2.30 (br, 1H, OH), 3.50−3.78 (m, 2H, 4-H, 5-H), 4.98 (br d, 1H, J = 7.8 Hz, NH), 5.07 (s, 2H, benzyl-H), 7.32 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 14.0 (11-C), 22.1, 23.1, 24.7 (1-C, 2-C, 2-CH_3), 22.5, 25.7, 29.2, 31.7, 34.2, 41.7 (3-C, 6-C, 7-C, 8-C, 9-C, 10-C), 52.8 (4-C), 66.6 (benzyl-C), 73.7 (5-C), 127.8, 127.9, 128.4 (arom CH), 136.6 (arom C), 156.7 (N−CO−O); MS *m*/*z* 336.2 ($M^+ + 1$).

4-[(Benzyloxycarbonyl)amino]-2-methyltridecan-5-ol (7f): yield 92%; IR (KBr/liquid film, cm^{-1}) 3339 (OH), 1685 (N−CO−O); ^{1}H NMR ($CDCl_3$) δ 0.80−0.98, 1.15−1.55, 1.57−1.72 (each m, 9H, 16H, 1H, aliph H), 2.19 (br, 1H, OH), 3.54−3.80 (m, 2H, 4-H, 5-H), 4.86 (br d, 1H, J = 8.4 Hz, NH), 5.09 (s, 2H, benzyl-H), 7.34 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 14.1 (13-C), 22.1, 23.2, 24.7 (1-C, 2-C, 2-CH_3), 22.6, 26.0, 29.2, 29.5, 29.7, 31.8, 33.1, 38.1 (3-C, 6-C, 7-C, 8-C, 9-C, 10-C,11-C, 12-C), 53.9 (4-C), 66.8 (benzyl-C), 74.8 (5-C), 128.0, 128.1, 128.5 (arom CH), 136.5 (arom C), 156.7 (N−CO−O); MS *m*/*z* 364.3 ($M^+ + 1$).

4-[(Benzyloxycarbonyl)amino]-3-methylnonan-5-ol (7g): yield 90%; IR (KBr/liquid film, cm^{-1}) 3337 (OH), 1688 (N−CO−O); ^{1}H NMR ($CDCl_3$) δ 0.80−0.97, 0.98−1.18, 1.20−1.64 (each m, 9H, 1H, 8H, aliph H), 2.30 (br, 1H, OH), 3.55−3.62, 3.62−3.68 (each m, 1H, 1H, 4-H, 5-H), 4.74 (br d, 1H, J = 8.7 Hz, NH), 5.09 (s, 2H, benzyl-H), 7.34 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 11.3, 14.0, 16.2 (1-C, 3-CH_3, 9-C), 22.7, 24.6, 28.1, 31.9 (2-C, 6-C, 7-C, 8-C) 35.4 (3-C), 60.5 (4-C), 66.9 (benzyl-C), 72.3 (5-C), 128.0, 128.1, 128.5 (arom CH), 136.4 (arom C), 157.3 (N−CO−O); MS *m*/*z* 308.2 ($M^+ + 1$).

4-[(Benzyloxycarbonyl)amino]-3-methylundecan-5-ol (7h): yield 94%; IR (KBr/liquid film, cm^{-1}) 3335 (OH), 1688 (N−CO−O); ^{1}H NMR ($CDCl_3$) δ 0.80−0.98, 0.98−1.18, 1.18−1.65 (each m, 9H, 1H, 12H, aliph H), 2.14 (br, 1H, OH), 3.55−3.62, 3.65−3.75 (each m, 1H, 1H, 4-H, 5-H), 4.69 (br d, 1H, J = 9.3 Hz, NH), 5.10 (s, 2H, benzyl-H), 7.34 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 11.3, 14.0, 16.2 (1-C, 3-CH_3, 11-C), 22.6, 24.7, 25.9, 29.3, 31.8, 32.2 (2-C, 6-C, 7-C, 8-C, 9-C, 10-C), 35.4 (3-C), 60.5 (4-C), 66.9 (benzyl-C), 72.4 (5-C), 128.0, 128.1, 128.5 (arom CH), 136.4 (arom C), 157.3 (N−CO−O); MS *m*/*z* 336.2 ($M^+ + 1$).

4-[(Benzyloxycarbonyl)amino]-3-methyltridecan-5-ol (7i): yield 90%; IR (KBr/liquid film, cm^{-1}) 3337 (OH), 1687 (N−CO−O); ^{1}H NMR ($CDCl_3$) δ 0.81−0.98, 0.98−1.18, 1.18−1.63 (each m, 9H, 1H, 16H, aliph H), 2.22 (br, 1H, OH), 3.55−

3.62, 3.63−3.73 (each m, 1H, 1H, 4-H, 5-H), 4.72 (br d, 1H, J = 9.6 Hz, NH), 5.09 (s, 2H, benzyl-H), 7.34 (s, 5H, arom H); ^{13}C NMR ($CDCl_3$) δ 11.3, 14.0, 16.2 (1-C, 3-CH_3, 13-C), 22.6, 24.6, 26.0, 29.2, 29.5, 29.7, 31.8, 32.2 (2-C, 6-C, 7-C, 8-C, 9-C, 10-C, 11-C, 12-C), 35.4 (3-C), 60.5 (4-C), 66.9 (benzyl-C), 72.3 (5-C), 128.0, 128.1, 128.5 (arom CH), 136.4 (arom C), 157.3 (N−CO−O); MS m/z 364.2 ($M^+ + 1$).

General Procedure for the Preparation of Amino Alcohols 8. The *N*-Cbz-protected amino alcohol **7** (3 mmol) was dissolved in methanol (20 mL, with addition of 0.1 mL of acetic acid) and hydrogenated with Pd/C (80 mg, 10%) as catalyst overnight. After filtration the solution was concentrated in vacuo, redissolved in dichloromethane, and washed with Na_2CO_3 solution (2 N, 2 × 20 mL) and brine (2 × 20 mL). Then, the solution was dried (Na_2SO_4) and evaporated under reduced pressure, yielding amino alcohols **8** as white solids.

4-Aminononan-5-ol (8a): yield 85%; ^{1}H NMR ($CDCl_3$) δ 0.80−0.95 (m, 6H, CH_3), 1.15−1.40 (m, 10H, CH_2), 2.42/2.57 (m, 1H, 4-H), 3.15/3.33 (m, 1H, 5-H), 3.80 (br, 3H, NH_2, OH); ^{13}C NMR ($CDCl_3$ + MeOD-d_3) δ 13.6, 13.7 (1-C, 9-C), 19.3, 22.4, 28.1, 31.0, 33.5 (2-C, 3-C, 6-C, 7-C, 8-C), 54.9 (4-C), 74.1 (5-C); MS m/z 160.2 ($M^+ + 1$).

4-Aminoundecan-5-ol (8b): yield 88%; ^{1}H NMR ($CDCl_3$ + MeOD-d_3) δ 0.64−0.80 (m, 6H, CH_3), 1.00−1.35 (m, 14H, CH_2), 2.38/2.53 (m, 1H, 4-H), 3.22/3.30 (m, 1H, 5-H), 4.18 (br, 3H, NH_2, OH); ^{13}C NMR ($CDCl_3$ + MeOD-d_3) δ 13.5, 13.6 (1-C, 11-C), 19.2, 22.2, 25.8, 29.0, 31.2, 31.5, 33.2 (2-C, 3-C, 6-C, 7-C, 8-C, 9-C, 10-C), 54.9 (4-C), 73.9 (5-C); MS m/z 188.2 ($M^+ + 1$).

4-Aminotridecan-5-ol (8c): yield 82%; ^{1}H NMR ($CDCl_3$ + MeOD-d_3) δ 0.62−0.78 (m, 6H, CH_3), 1.05−1.38 (m, 18H, CH_2), 2.40/2.51 (m, 1H, 4-H), 3.24/3.35 (m, 1H, 5-H), 4.10 (br, 3H, NH_2, OH); ^{13}C NMR ($CDCl_3$ + MeOD-d_3) δ 13.5, 13.6 (1-C, 13-C), 19.2, 22.2, 25.8, 29.0, 29.3, 29.8, 31.2, 31.5, 33.2 (2-C, 3-C, 6-C, 7-C, 8-C, 9-C, 10-C, 11-C, 12-C), 54.8 (4-C), 74.0 (5-C); MS m/z 216.2 ($M^+ + 1$).

4-Amino-2-methylnonan-5-ol (8d): yield 89%; ^{1}H NMR ($CDCl_3$ + MeOD-d_3) δ 0.60−0.85 (m, 9H, CH_3), 0.95−1.15, 1.22, 1.43 (each m, 7H, 1H, 1H, CH, CH_2), 2.43/2.58 (m, 1H, 4-H), 3.08/3.28 (m, 1H, 5-H), 4.18 (br, 3H, NH_2, OH); ^{13}C NMR ($CDCl_3$ + MeOD-d_3) δ 13.3 (9-C), 21.0, 23.0, 24.1 (1-C, 2-C, 2-CH_3), 22.2, 27.9, 30.8, 33.7 (3-C, 6-C, 7-C, 8-C), 52.8 (4-C), 73.6 (5-C); MS m/z 173.2 (M^+).

4-Amino-2-methylundecan-5-ol (8e): yield 87%; ^{1}H NMR ($CDCl_3$ + MeOD-d_3) δ 0.60−0.85 (m, 9H, CH_3), 0.90−1.15, 1.25, 1.45 (each m, 11H, 1H, 1H, CH, CH_2), 2.45/2.58 (m, 1H, 4-H), 3.05/3.27 (m, 1H, 5-H), 4.20 (br, 3H, NH_2, OH); ^{13}C NMR ($CDCl_3$ + MeOD-d_3) δ 13.2 (11-C), 20.9, 23.0, 24.0 (1-C, 2-C, 2-CH_3), 22.2, 25.8, 29.0, 31.2, 31.5, 33.2 (3-C, 6-C, 7-C, 8-C, 9-C, 10-C), 52.9 (4-C), 73.8 (5-C); MS m/z 201.2 (M^+).

4-Amino-2-methyltridecan-5-ol (8f): yield 88%; ^{1}H NMR ($CDCl_3$) δ 0.78−0.98 (m, 9H, CH_3), 1.12−1.36, 1.44, 1.65 (each m, 15H, 1H, 1H, CH, CH_2), 2.58/2.80 (m, 1H, 4-H), 3.24/3.42 (m, 1H, 5-H), 4.18 (br, 3H, NH_2, OH); ^{13}C NMR ($CDCl_3$ + MeOD-d_3) δ 13.3 (9-C), 20.9, 22.9, 24.1 (1-C, 2-C, 2-CH_3), 22.6, 25.7, 29.2, 29.5, 31.2, 31.8, 33.1, 34.3 (3-C, 6-C, 7-C, 8-C, 9-C, 10-C, 11-C, 12-C), 53.0 (4-C), 73.5 (5-C); MS m/z 229.2 (M^+).

4-Amino-3-methylnonan-5-ol (8g): yield 90%; ^{1}H NMR ($CDCl_3$) δ 0.83−0.90 (m, 9H, CH_3), 1.12−1.42, 1.53, 1.65 (each m, 9H, CH, CH_2), 2.38/2.48 (m, 1H, 4-H), 3.45/3.58 (m, 1H, 5-H); ^{13}C NMR ($CDCl_3$) δ 10.8, 14.1, 15.6 (1-C, 2-CH_3, 9-C), 22.8, 25.4, 28.4, 29.8 (2-C, 6-C, 7-C, 8-C), 37.7 (3-C), 60.2 (4-C), 71.4 (5-C); MS m/z 174.2 ($M^+ + 1$).

4-Amino-3-methylundecan-5-ol (8h): yield 92%; ^{1}H NMR ($CDCl_3$) δ 0.85−0.96 (m, 9H, CH_3), 1.15−1.45, 1.55, 1.60−1.70 (each m, 13H, CH, CH_2), 2.38/2.48 (m, 1H, 4-H), 3.45/3.60 (m, 1H, 5-H); ^{13}C NMR ($CDCl_3$) δ 10.8, 14.1, 15.6 (1-C, 2-CH_3, 11-C), 22.6, 25.5, 26.1, 29.4, 30.1, 31.9 (2-C, 6-C, 7-C, 8-C, 9-C, 10-C), 37.8 (3-C), 60.3 (4-C), 71.3 (5-C); MS m/z 202.2 ($M^+ + 1$).

4-Amino-3-methyltridecan-5-ol (8i): yield 91%; ^{1}H NMR ($CDCl_3$) δ 0.82−0.97 (m, 9H, CH_3), 1.10−1.44, 1.55, 1.65 (each m, 17H, CH, CH_2), 2.36/2.48 (m, 1H, 4-H), 3.43/3.60 (m, 1H, 5-H); ^{13}C NMR ($CDCl_3$) δ 10.7, 14.0, 15.6 (1-C, 2-CH_3, 13-C), 22.5, 23.8, 25.3, 26.1, 29.2, 29.6, 30.0, 31.8 (2-C, 6-C, 7-C, 8-C, 9-C, 10-C, 11-C, 12-C), 38.5 (3-C), 61.2 (4-C), 72.3 (5-C); MS m/z 230.2 ($M^+ + 1$).

General Procedure for Amide Formation yielding Compounds 9. A mixture of N-deprotected amino alcohol **8** (1 mmol) and Boc-Val-Val-OH (1 mmol, 320 mg) in a solvent mixture of tetrahydrofuran and dichloromethane was treated with EEDQ (2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline) (1.2 mmol, 300 mg) and stirred at room temperature for 24 h. Then, the solution was evaporated, redissolved in ethyl acetate, and washed with HCl (1 N, 2 × 10 mL), saturated aqueous $NaHCO_3$ solution (2 × 20 mL), and brine (2 × 20 mL). The organic phase was dried (Na_2SO_4) and evaporated. Flash chromatography of the residue (ethyl acetate−hexane; 1:1) afforded the pure products.

4-[*N*-[(*tert*-Butoxycarbonyl)-L-valyl-L-valyl]amino]-nonan-5-ol (9a): yield 63%; R_f 0.45; HRMS 457.352 (M^+), calcd 457.352 for $C_{24}H_{47}N_3O_5$.

4-[*N*-[(*tert*-Butoxycarbonyl)-L-valyl-L-valyl]amino]undecan-5-ol (9b): yield 60%; R_f 0.45; IR (KBr/liquid film, cm^{-1}) 3328 (NH, OH), 1716 (N−CO−O), 1660 (CO−NH); ^{1}H NMR ($CDCl_3$ + MeOD-d_3) δ 0.80−0.92, 1.05−1.15, 1.15−1.40 (m, 32H, chain-CH_2, CH_3), 1.42 [s, 9H, $C(CH_3)_3$], 2.13, 2.18 [each m, 1H, 1H, C*H*$(CH_3)_2$], 3.52, 4.08, 4.25, 4.90 (each m, α-H, 4-H, 5-H), 5.25, 6.85, 7.30 (3 × br d, NH, OH); HRMS 485.383 (M^+), calcd 485.383 for $C_{26}H_{51}N_3O_5$.

4-[*N*-[(*tert*-Butoxycarbonyl)-L-valyl-L-valyl]amino] tridecan-5-ol (9c): yield 68%; R_f 0.50; HMRS 513.414 (M^+), calcd 513.414 for $C_{28}H_{55}N_3O_5$.

4-[*N*-[(*tert*-Butoxycarbonyl)-L-valyl-L-valyl]amino]-2-methylnonan-5-ol (9d): yield 65%; R_f 0.45; HMRS 471.367 (M^+), calcd 471.367 for $C_{25}H_{49}N_3O_5$.

4-[*N*-[(*tert*-Butoxycarbonyl)-L-valyl-L-valyl]amino]-2-methylundecan-5-ol (9e): yield 68%; R_f 0.55; IR (KBr/liquid film, cm^{-1}) 3370 (NH, OH), 1720 (N−CO−O), 1670 (CO−NH); ^{1}H NMR ($CDCl_3$) δ 0.80−0,95 (m, 21H, CH_3), 1.15−1.48 (m, 12H, chain-CH_2), 1.41 [s, 9H, $C(CH_3)_3$], 2.19, 2.38 [each m, 1H, 1H, C*H*$(CH_3)_2$], 3.33, 3.48, 3.95, 4.37 (each m, α-H, 4-H, 5-H), 5.40, 6.90, 7.20 (3 × br d, NH, OH); HRMS m/z 499.399 (M^+), calcd 499.399 for $C_{27}H_{53}N_3O_5$.

4-[*N*-[(*tert*-Butoxycarbonyl)-L-valyl-L-valyl]amino]-2-methyltridecan-5-ol (9f): yield 70%; R_f 0.60; HRMS m/z 527.430 (M^+), calcd 527.430 for $C_{29}H_{57}N_3O_5$.

4-[*N*-[(*tert*-Butoxycarbonyl)-L-valyl-L-valyl]amino]-3-methylnonan-5-ol (9g): yield 58%; R_f 0.55; HRMS m/z 471.367 (M^+), calcd 471.367 for $C_{25}H_{49}N_3O_5$.

4-[*N*-[(*tert*-Butoxycarbonyl)-L-valyl-L-valyl]amino]-3-methylundecan-5-ol (9h): yield 60%; R_f 0.60; IR (KBr/liquid film, cm^{-1}) 3436 (NH, OH), 1718 (N−CO−O), 1670 (CO−NH); ^{1}H NMR ($CDCl_3$ + MeOD-d_3) δ 0.70−1.05, 1.10−1.28 (m, 32H, chain-CH_2, CH_3), 1.42 [s, 9H, $C(CH_3)_3$], 2.06, 2.20 [each m, 1H, 1H, C*H*$(CH_3)_2$], 4.00, 4.32, 4.90, 4.99 (each m, α-H, 4-H, 5-H), 7.20 (br d, NH); HRMS m/z 499.398, calcd 499.398 for $C_{27}H_{53}N_3O_5$.

4-[*N*-[(*tert*-Butoxycarbonyl)-L-valyl-L-valyl]amino[-3-methyltridecan-5-ol (9i): yield 63%; R_f 0.60; HRMS m/z 527.430 (M^+), calcd 527.430 for $C_{29}H_{57}N_3O_5$.

Enzyme Assay. Pepsin inhibition studies of all compounds (**3**−**9**) were carried out in a modified way as described previously by Bernkop-Schnürch and Dundalek.[7] First, 1.00 mg of the test compound was dissolved in 500 μL of dimethyl sulfoxide (DMSO) and the solution diluted in 1:10 (v:v) steps with the same solvent. The 100 L of an appropriate dilution was transferred to the first well of a microtitration plate (96-well, not binding) and diluted in 1:2 (v:v) steps with DMSO in the following five vertical wells. To each 50 μL of diluted test compound, 75 μL of a fresh prepared pepsin solution [1 mg of pepsin (40 units; Sigma, St. Louis, MO) per mL of 0.05 N HCl] were added. Next, 37.5 μg of horseradish peroxidase (Sigma) in 75 μL of 0.05 N HCl was transferred to each well, and reaction mixtures were incubated for 2 h at 37 °C. Thereafter, samples were diluted in 1:2 (v:v) steps with 0.05 N HCl in the following eleven horizontal wells. To each 100 μL of diluted reaction mixture was added 100 μL of the substrate medium (24 mg of *o*-phenylenediamine dihydrochloride, 12 mL of 0.2

M phosphate buffer pH 6.5, and 24 μL of 30% H_2O_2), and the enzymatic reaction was allowed to proceed at room temperature for 10 min. Optical density was read at 492 nm with a microtitration plate reader. Reaction mixtures containing increasing pepstatin concentrations instead of test compounds were used as references. For negative and positive controls, the enzyme assay was carried out as described above, but omitting test compounds or test compounds as well as pepsin, respectively.

Statistical Data Analysis. Statistical data analysis were performed using the *t*-test with $p < 0.05$ as the minimal level of significance.

References

(1) Bernkop-Schnürch, A. The use of inhibitory agents to overcome the enzymatic barrier to perorally administered therapeutic peptides and proteins. *J. Control. Release*, in press.
(2) Bernkop-Schnürch, A.; Schwarz, G. H.; Kratzel, M. Modified Mucoadhesive Polymers for the Peroral Administration of Mainly Elastase Degradable Therapeutic (Poly)peptides. *J. Control. Release* **1997**, *47*, 113−121.
(3) Bernkop-Schnürch, A.; Bratengeyer, I.; Valenta, C. Development of a drug delivery system protecting from trypsinic degradation. *Int. J. Pharm.* **1997**, *157*, 17−25.
(4) Cho, Y.-K.; Rebholz, K. L.; Northrop, D. B. Solvent Isotope Effects on the Onset of Inhibition of Porcine Pepsin by Pepstatin. *Biochemistry* **1994**, *33*, 9637−9642.
(5) Itoh, M.; Matsuo, Y. Gastric ulcer treatment with intravenous human epidermal growth factor: A double-blind controlled clinical study. *J. Gastroenterol. Hepatol.* **1994,** *9*, 78−83.
(6) Slomiany, B. L.; Nishikawa, H.; Bilski, J.; Slomiany, A. Colloidal Bismuth Subcitrate Inhibits Peptic Degradation of Gastric Mucus and Epidermal Growth Factor *in Vitro*. *Am. J. Gastroenterol.* **1990**, *85*, 390−393.
(7) Bernkop-Schnürch, A.; Dundalek, K. Novel bioadhesive drug delivery system protecting (poly)peptides from gastric enzymatic degradation. *Int. J. Pharm.* **1996**, *138*, 75−83.
(8) Carmel, R. In vitro studies of gastric juice in patients with food-cobalamin malabsorption. *Digest. Dis. Sci.* **1994**, *39*, 2516−2522.
(9) McCaffrey, G.; Jamieson, J. C. Evidence for the role of a cathepsin D-like activity in the release of gal β1-4 glcnac α2-6 sialyltransferase from rat and mouse l liver in whole-cell systems. *Comp. Biochem. Phys. C.* **1993**, *104*, 91−94.
(10) Plumpton, C.; Kalinka, S.; Martin, R. C.; Horton, J. K.; Davenport, A. P. Effects of phosphoramidon and pepstatin A on the secretion of endothelin-1 and big endothelin-1 by human umbilical vein endothelial cells—measurement by two-site enzyme-linked immunosorbent assays. *Clin. Sci.* **1994**, *87*, 245−251.
(11) McConnell, R. M.; Frizzell, D.; Evans, A. C. A.; Jones, W.; Cagle, C. New Pepstatin Analogues: Synthesis and Pepsin Inhibition. *J. Med. Chem.* **1991**, *34*, 2298−2300.
(12) Basha, A.; Lipton, M.; Weinreb, S. M. *Tetrahedron Lett.* **1977**, *48*, 4171−4172.
(13) Kano, S.; Yokomatsu, T.; Iwasawa, H.; Shibuya, S. *Tetrahedron Lett.* **1987**, *28*, 6331− 6334 and literature cited therein.

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