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A Simple and Convenient Method for the Synthesis of Iminomethylene Analogs of Peptides

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The synthesis of pseudopeptides in which a part of the peptide bonds has been replaced by other isosteric bonds has been studied in order to obtain long-lasting peptide drugs and also to study the structure-activity relationships of peptides with biological activity. A simple and convenient method for the synthesis of pseudo[CH_2–NH]peptides was established by improving the conditions for the selective reduction of the peptide bond of *N*-protected dipeptide esters by borane. Among the ester residues investigated, the *t*-butyl ester gave the best result. During reactions no racemization occurred. [L-Pheψ[CH_2–NH]L-Leu$^{4-5}$]leucine-enkephalin was synthesized in good yield starting from *N*-benzyloxycarbonyl–L-phenylalanyl–L-leucine *t*-butyl ester.

Numbers of biologically active peptides which can be isolated or the sequences of which are determined by gene technical methods have been increasing. In the study of their physiological roles and their applications as drugs, native peptides have a substantial restriction due to their rapid degradation by peptidase. As a method for increasing the stability of peptides towards proteolytic enzymes, (and at the same time to determine the contribution of a specific amino acid residue to the biological activity,) syntheses of analogues with some residues replaced by unnatural amino acids such as *N*-methyl amino acids or D-amino acids have been tried with success.

As another approach to this problem, the syntheses of pseudopeptides in which a specific peptide bond is replaced by an appropriate isosteric bond stable to hydrolysis (such as CH=CH, CH_2–CO, CH_2–CH_2, CH_2–S, or CH_2–NH) have been studied. Among these isosteric bonds, the CH_2–NH bond has the characteristic that it maintains the amine function with a capacity as a hydrogen-bond donor. However, ψ[CH_2–NH]peptides have so far not been widely utilized since methods for the introduction of this bond to any desired position in a sequence of peptide have not been established. This study offers the first general method for the preparation of an *N*-protected ψ[CH_2–NH]dipeptide acid unit necessary for the synthesis of ψ[CH_2–NH]peptides.

The synthesis of ψ[CH_2–NH]peptides have been approached from two routes: 1) by direct CH_2–NH bond formation from a peptide aldehyde and a peptide ester, either through a Schiff-base formation followed by hydrogenation[1,2] or by a reductive alkylation[3] and 2) by the incorporation of a protected ψ[CH_2–NH]dipeptide acid unit by a usual peptide synthetic method.[4] The ψ[CH_2–NH]dipeptide acid units have been prepared from 2-(protected amino)ethyl tosylate[4] or halo acid[5] by a substitution reaction, or from protected dipeptide esters (either by the selective reduction of the peptide bond by borane followed by hydrolysis[6] or by reduction by sodium bis(2-methoxyethoxy)aluminium hydride followed by oxidation[4]). Most of these routes, however, can not be the general method owing to the complexity of the reactions. Only one exception, a reduction by borane, has a substantial possibility to be a general method. However, the procedure proposed by Roeske's group is not practically applicable because of the poor yield and difficulty of separation of the desired product from the by-products.[4] In this study we reinvestigated the conditions for a reduction of protected dipeptide esters by borane in order to establish a general method for the incorporation of the CH_2–NH bond in any desired position of the peptides.

A retention of asymmetry at chiral centers during the reduction and the necessity of protecting the generated secondary amine function during peptide bond elongation steps are also discussed.

Results and Discussion

Borane is known to reduce amide bonds selectively, but in the case of a protected dipeptide ester, a reduction of the ester moiety often accompanies this reaction. Roeske's group reported that a concurrent reduction of the ester bond was minimized by carrying out the reaction at −20 °C with 2 mol of BH_3 for 4—5 h. However, the fact that such selectivity is obtained only in a low conversion-stage restricted the practical application of their procedure. Then, we examined in detail the conditions for the reduction of protected dipeptide esters by borane and the effects of ester residues on the product yields.

Optimization of the conditions of the reduction of protected dipeptide esters by borane was tried by using *N*-benzyloxycarbonyl(Z)-L-phenylalanyl-L-Phenylalanine 2-methoxyethyl ester (**1a**) as a model compound.

$$X\text{-}AA^1\text{-}AA^2\text{-}CO_2Y \xrightarrow{BH_3\text{-}THF}$$
(**1a—g**)

$$X\text{-}AA^1\psi[CH_2\text{-}NH]AA^2\text{-}CO_2Y + X\text{-}AA^1\text{-}AA^2\text{-}CH_2OH$$
(**2a—g**) (**3a,b,e—g**)

$$+ X\text{-}AA^1\psi[CH_2\text{-}NH]AA^2\text{-}CH_2OH$$
(**4a,b,e—g**)

a: X=Z, AA^1=L-Phe, AA^2=L-Phe, Y=$CH_2CH_2OCH_3$
b: X=Z, AA^1=L-Phe, AA^2=L-Leu, Y=$CH_2CH_2OCH_3$
c: X=Z, AA^1=L-Phe, AA^2=L-Leu, Y=CH_3
d: X=Z, AA^1=L-Phe, AA^2=L-Leu, Y=$C(CH_3)_3$
e: X=Z, AA^1=L-Phe, AA^2=D-Leu, Y=$C(CH_3)_3$
f: X=Z, AA^1=Gly, AA^2=L-Phe, Y=$C(CH_3)_3$
g: X=Boc, AA^1=L-Phe, AA^2=L-Leu, Y=CH_3

Borane–tetrahydrofuran (THF) was added to a solution of **1a** in THF at −20 °C and then the temperature was increased to the point to be studied. After having been stirred at that temperature for an appropriate period, the remaining borane was quenched by the addition of methanol and the mixture was further treated with 1 M† HCl for 15 min to destroy boron–nitrogen bonds in products. Then, products were separated and isolated by preparative thin-layer chromatography (TLC) on silica gel. The results are summarized in Table 1 together with the results of a reduction of various kinds of protected dipeptide esters.

When the reaction temperature was raised between −5 and 0 °C, the yield of the corresponding pseudopeptide ester **2a** increased, while some part of it was further reduced to the pseudopeptide alcohol **4a** (Entries 3—5). In this case, the product of the selective reduction of the ester moiety, peptide alcohol **3a**, was not obtained. When the temperature was further raised to 30 °C, no further improvement in the conversion ratio of the starting material and yield of the desired product was obtained (Entry 6). Thus, the highest yield of the protected pseudodipeptide ester was obtained when a reduction of the protected dipeptide ester was carried out by use of 10 mol of BH_3 at 0 °C for 10 h (Entry 5).

In Entries 7—9 the effects of ester residues on the yield of the products were shown. The structure of the ester moiety has an important role in determining the ratio of the products. As expected, *t*-butyl ester (Entry 9) gave the highest yield of the desired pseudopeptide ester. However, a low yield was encountered in the case of Z-Gly-L-Phe-OBu^t, reflecting the lower reactivity of BH_3 toward α-unsubstituted amides[7] (Entry 11). On the other hand, these conditions are also applicable to *N*-*t*-butoxycarbonyl-dipeptide ester (Entry 12).

Racemization during the reduction was checked for two pseudopeptides, Z-L-Pheψ[CH_2-NH]L-Leu-OBu^t (**2d**) and Z-L-Pheψ[CH-NH]D-Leu-OBu^t (**2e**). Compounds **2d** and **2e** were deprotected by HBr in acetic acid and the free pseudodipeptides obtained were analyzed by reverse-phase HPLC.[8] Both compounds were eluted separately, and L-Pheψ[CH_2-NH]D-Leu was not detected in a sample of synthesized L-Pheψ[CH_2-NH]L-Leu. Since D-Pheψ[CH_2-NH]L-Leu, a mirror image of the L-Pheψ[CH_2-NH]D-Leu, should be eluted at the same position as L-Pheψ[CH_2-NH]D-Leu it can be concluded that racemization does not occur during the reduction at both chiral centers of the dipeptides.

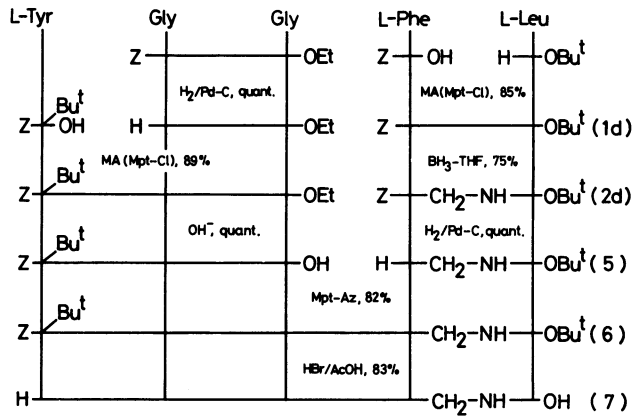
In planning the synthesis of ψ[CH_2-NH]peptides by utilizing the ψ[CH_2-NH]dipeptide unit thus obtained, the necessity of protecting the secondary amine function should be considered. Most of the syntheses used so far protecting groups,[4] but syntheses without protection have also been reported.[1,2] In order to clarify this point and at the same time to determine the effect of a displacement of the peptide bond by an isosteric bond on the biological activity, we synthesized a pseudopeptide analogue of Leu-

Table 1. Reduction of *N*-Protected Dipeptide Esters **1a—g** by Borane

Entry	Compound	Reaction Conditions			Yield/%[a]			Recovery/%[a]
		Temp/°C	BH_3/**1** (mol mol^{-1})	Time/h	**2**	**3**	**4**	**1**
1	**1a**	−10	10	5	24	0	0	66
2	**1a**	−5	5	2	47	0	0	43
3	**1a**	−5	5	10	61	0	0	28
4	**1a**	0	5	10	60	0	7	21
5	**1a**	0	10	10	69	0	13	11
6	**1a**	30	5	1	41	0	12	10
7	**1b**	0	10	10	58	5	9	ND
8	**1c**	0	10	10	64	7	10	ND
9	**1d**	0	10	10	75	ND	5	ND
10	**1e**	0	10	10	49	ND	ND	ND
11	**1f**	0	10	10	16	ND	ND	ND
12	**1g**	0	10	10	47	ND	ND	ND

a) Isolated yield. ND: not determined.

† 1 M=1 mol dm^{-3}.



Scheme 1. Synthesis of [L-Pheψ[CH_2–NH]L-Leu$^{4-5}$]-leucine-enkephalin.

enkephalin, in which a peptide bond between Phe^4 and Leu^5 residues was replaced by the CH_2–NH bond. The synthetic route is shown in Scheme 1.

The benzyloxycarbonyl group of **2d** was removed by catalytic hydrogenolysis to give H-L-Pheψ[CH_2–NH]L-Leu-OBu^t (**5**) in quantitative yield as dihydrochloride. The coupling of **5** with a protected *N*-terminal tripeptide acid unit was carried out with the use of dimethylphosphinothioic azide as a coupling reagent[9,10] to afford the desired protected pseudopentapeptide ester, Z-L-Tyr(Bu^t)-Gly-Gly-L-Pheψ-[CH_2–NH]L-Leu-OBu^t (**6**), in 82% yield. In this reaction no significant amount of side products could be detected, showing that the protection of the secondary amine function was not necessary. The removal of all the protecting groups by HBr in acetic acid, followed by purification by preparative TLC and gel chromatography on Sephadex LH-20 gave chromatographically homogeneous [L-Pheψ[CH_2–NH]L-Leu$^{4-5}$]leucine-enkephalin (**7**) in 83% yield.

Measurements of the pharmacological activity of **7** in in vitro isolated tissue preparations are now being studied. Results will be reported elsewhere.

Experimental

All the melting points were uncorrected. TLC was performed on silica-gel plates (Merck 60 GF_{254}) in the following solvent systems(v/v): chloroform–methanol (9:1, R_f^1), chloroform–methanol (49:1, R_f^2), ether–petroleum ether (1:1, R_f^3), ethyl acetate (R_f^4), chloroform–methanol–water (65:25:4, R_f^5), chloroform–methanol–acetic acid (85:25:20, R_f^6), chloroform–methanol–acetic acid (95:5:3, R_f^7), 1-butanol–acetic acid–water (4:1:1, R_f^8). Diborane solution in THF was prepared from $NaBH_4$ and boron trifluoride etherate and standardized before use. For most of the experiments the concentration was approximately 2 M in BH_3. All the stepwise syntheses of protected di- and tripeptides were mediated with the mixed anhydride method with the use of dimethylphosphinothioic chloride (Mpt-Cl) as the coupling reagent.[11]

H-L-Phe-$OCH_2CH_2OCH_3$·HCl (8) and H-L-Leu-$OCH_2CH_2OCH_3$·HCl (9): These compounds were prepared from the corresponding amino acids and 2-methoxyethanol by the usual method using thionyl chloride. **8**: Mp 173.0—175.6 °C; $[\alpha]_D^{22}$ +15.7° (*c* 2.0, abs. EtOH); **9**: Mp 124.8—125.2 °C; $[\alpha]_D^{22}$ +12.5° (*c* 2.0, abs. EtOH).

Z-L-Phe-L-Phe-$OCH_2CH_2OCH_3$ (1a): The Mpt-Cl (2.57 g, 20 mmol) was added at rt to a stirred mixture of Z-L-Phe-OH (5.99 g, 20 mmol) and triethylamine (2.79 ml, 20 mmol) in chloroform (20 ml). After 30 min, to this solution, **8** (5.20 g, 20 mmol) in chloroform (20 ml) and triethylamine (5.58 ml, 40 mmol) were added at 0 °C and the solution was stirred at rt for 11 h. All the volatile materials were removed in vacuo. The residue was taken in ethyl acetate and the solution washed in the usual manner and dried. After evaporation of the solvent, the crude material was recrystallized from ethyl acetate–petroleum ether. Yield 8.57 g (85%); mp 119.5—120.0 °C; $[\alpha]_D^{27}$ +24.8° (*c* 1.0, $CHCl_3$); R_f^2=0.60, R_f^3=0.10; ^{1}H NMR ($CDCl_3$) δ=2.9—3.2 (m, 4H), 3.3—3.7 (m, 5H), 4.1—4.9 (m, 4H), 5.0 (s, 2H), 5.3—5.6 (d, 1H), 6.3—6.7 (d, 1H), 6.9—7.4 (aromatic protons, 15H).

Found: C, 68.71; H, 6.36; N, 5.40%. Calcd for $C_{29}H_{32}O_6N_2$: C, 69.03; H, 6.39; N, 5.55%.

Z-L-Phe-L-Leu-$OCH_2CH_2OCH_3$ (1b): This compound was obtained using **9** in the same way as described above. Crude products were recrystallized from ether–petroleum ether. Yield 62%; mp 77.0—77.8 °C; $[\alpha]_D^{27}$ −4.7° (*c* 1.0, $CHCl_3$); R_f^2=0.55, R_f^3=0.13; ^{1}H NMR($CDCl_3$) δ=0.8—1.0 (d, 6H), 1.4—1.9 (m, 3H), 3.0—3.2 (d, 2H), 3.35 (s, 3H), 3.5—3.7 (t, 2H), 4.1—4.8 (m, 4H), 5.07 (s, 2H), 5.2—5.5 (broad, 1H), 6.1—6.6 (broad, 1H), 7.1—7.4 (aromatic protons, 10H).

Found: C, 66.22; H, 7.24; N, 5.96%. Calcd for $C_{26}H_{34}O_6N_2$: C, 66.36; H, 7.28; N, 5.95%.

General Procedure for the Reduction of *N*-Protected Dipeptide Esters by BH_3–THF (Table 1): Borane–THF solution (2.5—5.0 mmol) was slowly added to the stirred solution of **1** (0.5 mmol) in THF at −20 °C. The reaction mixture was stirred at an appropriate temperature for an appropriate period (see Table 1). The reaction was quenched by the addition of methanol (5 ml) and then 1 M HCl (5 ml). After stirring for 15 min, the pH of the mixture was adjusted to 7—8 by a 5% aq $NaHCO_3$ solution. After removing THF in vacuo the desired product was extracted with chloroform or ethyl acetate. The combined extracts were washed, dried and evaporated under a reduced pressure, and the residue was purified by preparative silica-gel TLC.

Reduction of Z-L-Phe-L-Phe-$OCH_2CH_2OCH_3$ (1a): The reaction was carried out according to the general procedure. The purification by TLC was performed twice using different solvent systems; ether–petroleum ether (2:1 v/v) and then chloroform–methanol (20:1 v/v). **2a** was obtained as pale-yellow crystals; mp 63.0—63.2 °C; $[\alpha]_D^{26}$ +15.2° (*c* 2.6, $CHCl_3$); R_f^2=0.57, R_f^3=0.17; ^{1}H NMR ($CDCl_3$) δ=1.77 (broad, 1H), 2.15—3.1 (m, 6H), 3.1—4.4 (m, 9H), 4.9—5.3 (m, 3H), 6.8—7.4 (aromatic protons, 15H).

Found: C, 70.88; H, 6.97; N, 5.69%. Calcd for $C_{29}H_{34}O_5N_2$: C, 71.00; H, 6.99; N, 5.71%.

4a was obtained as colorless crystals. An analytical sample was recrystallized from ethyl acetate–petroleum ether; mp 111.0—111.7 °C; $[\alpha]_D^{26}$ +16.0° (*c* 0.6, $CHCl_3$); R_f^1=

0.49, R_f^5=0.38; ^{1}H NMR ($CDCl_3$) δ=2.25 (broad, 2H), 2.5—2.9 (m, 7H), 3.1—4.2 (m, 3H), 4.9—5.3 (m, 3H), 6.9—7.4 (aromatic protons, 15H).

Found: C, 74.32; H, 7.21; N, 6.57%. Calcd for $C_{26}H_{30}O_3N_2$: C, 74.61; H, 7.23; N, 6.69%.

Reduction of Z-L-Phe-L-Leu-OCH$_2$CH$_2$OCH$_3$ (1b): The reaction of **1b** (235 mg, 0.5 mmol) was carried out according to the general procedure. Purification by TLC was performed twice using different solvent systems; ether–petroleum ether (2:1 v/v) and then chloroform–methanol (10:1 v/v) to give **2b** as pale-yellow crystals; mp 54.0—55.0 °C; $[\alpha]_D^{19}$ −16.2° (*c* 1.0, $CHCl_3$); R_f^2=0.56, R_f^3=0.25; ^{1}H NMR ($CDCl_3$) δ=0.8—1.1 (d, 6H), 1.3—1.9 (m, 4H), 2.3—3.0 (m, 4H), 3.1—4.3 (m, 9H), 5.0—5.3 (m, 3H), 7.1—7.4 (aromatic protons, 10H).

Found: C, 67.94; H, 7.88; N, 5.92%. Calcd for $C_{26}H_{36}O_5N_2$: C, 68.39; H, 7.95; N, 6.14%.

3b (10.4 mg, 5.2%) was identical with the authentic sample prepared by the reduction of Z-L-Phe-L-Phe-OMe by $NaBH_4$; mp 119.0—121.3 °C; R_f^1=0.69, R_f^4=0.74.

4b (18.7 mg, 9.7%) was obtained as colorless crystals; mp 100.7—102.0 °C; R_f^1=0.44; R_f^4=0.13; ^{1}H NMR ($CDCl_3$) δ=0.7—1.0 (m, 6H), 1.0—1.9 (m, 3H), 2.1—4.4 (m, 10H), 5.0—5.3 (m, 3H), 7.1—7.6 (aromatic protons, 10H).

Reduction of Z-L-Phe-L-Leu-OMe (1c): According to the general procedure, **1c** (213 mg, 0.5 mmol) was reduced. **2c** was obtained as colorless crystals; 133 mg (64%); mp 66.0—68.0 °C; $[\alpha]_D^{19}$ −16.7° (*c* 1.0, $CHCl_3$); R_f^2=0.68; R_f^3=0.44; ^{1}H NMR ($CDCl_3$) δ=0.8—1.1 (d, 6H), 1.2—2.0 (m, 4H), 2.2—3.4 (m, 5H), 3.6—4.2 (m, 4H), 5.0—5.2 (m, 3H), 7.0—7.4 (aromatic protons, 10H).

Found: C, 69.72; H, 7.74; N, 6.48%. Calcd for $C_{24}H_{32}O_4N_2$: C, 69.88; H, 7.82; N, 6.79%.

Reduction of Z-L-Phe-L-Leu-OBu^t (1d): According to the general procedure, **1d** (217 mg, 0.5 mmol) was reduced. **2d** was obtained as colorless oil (158 mg, 75%); $[\alpha]_D^{19}$ −13.4° (*c* 1.1, $CHCl_3$); R_f^2=0.70; R_f^3=0.48.

Found: C, 70.47; H, 8.33; N, 5.76%. Calcd for $C_{27}H_{38}O_4N_2 \cdot 1/3H_2O$: C, 70.40; H, 8.46; N, 6.08%.

The acetic acid salt of **2d** was obtained as colorless crystals: Mp 76.8—78.6 °C; $[\alpha]_D^{27}$ −7.5° (*c* 1.0, $CHCl_3$).

Found: C, 67.47; H, 8.20; N, 5.47%. Calcd for $C_{29}H_{42}O_6N_2$: C, 67.68; H, 8.23; N, 5.44%.

Reduction of Z-L-Phe-D-Leu-OBu^t (1e): According to the general procedure, **1e** (141 mg, 0.30 mmol) was reduced. TLC purification in the solvent system of ether–petroleum ether (2:3 v/v) gave **2e** as a pale-yellow oil; 67.3 mg (49%); $[\alpha]_D^{25}$ +5.3° (*c* 3.8, $CHCl_3$); R_f^2=0.69; R_f^3=0.46. ^{1}H NMR ($CDCl_3$) δ=0.8—1.1 (d, 6H), 1.2—1.9 (m, 13H), 2.3—3.2 (m, 5H), 3.6—4.1 (broad, 1H), 5.0—5.3 (m, 3H), 7.1—7.4 (aromatic protons, 10H).

Found: C, 71.29; H, 8.37; N, 6.29%. Calcd for $C_{27}H_{38}O_4N_2$: C, 71.33; H, 8.43; N, 6.16%.

Reduction of Z-Gly-L-Phe-OBu^t (1f): According to the general procedure, **1f** (170 mg, 0.41 mmol) was reduced. TLC purification using ether as a solvent gave colorless oil of **2f**; 27.2 mg (17%); $[\alpha]_D^{27}$ +6.9° (*c* 3.0, methanol); R_f^1=0.80; R_f^5=0.56. ^{1}H NMR ($CDCl_3$) δ=1.35 (s, 9H), 1.65 (s, 1H), 2.6—3.6 (m, 7H), 5.0—5.3 (m, 3H), 7.1—7.4 (aromatic protons, 10H).

Found: C, 69.07; H, 7.56; N, 7.13%. Calcd for $C_{23}H_{30}O_4N_2$: C, 69.32; H, 7.59; N, 7.03%.

Reduction of Boc-L-Phe-L-Leu-OMe (1g): According to the general procedure, **1g** (157 mg, 0.40 mmol) was reduced. TLC purification using ether then chloroform–methanol (20:1 v/v) gave **2g** as colorless crystals; 71.7 mg (47%); mp 83.0—85.0 °C; $[\alpha]_D^{26}$ −2.3° (*c* 2, $CHCl_3$); R_f^2=0.63, R_f^3=0.48; ^{1}H NMR ($CDCl_3$) δ=0.6—1.1 (m, 6H), 1.4—2.0 (m, 13H), 2.2—3.5 (m, 5H), 3.5—4.3 (m, 4H), 4.4—4.9 (broad, 1H), 7.2 (aromatic protons, 5H).

Found: C, 66.58; H, 9.00; N, 7.57%. Calcd for $C_{21}H_{34}O_4N_2$: C, 66.63; H, 9.05; N, 7.40%.

Preparation of Z-L-Tyr(Bu^t)-Gly-Gly-L-Pheψ[CH$_2$-NH]L-Leu-OBu^t (6): Compound **2d** (145 mg, 0.32 mmol) was hydrogenolyzed in the presence of 10% Pd-C (50 mg) in methanol (4 ml) containing conc HCl (0.1 ml) to give 125.6 mg (quantitative) of H-L-Pheψ[CH$_2$-NH]L-Leu-OBu^t·2HCl, which was dissolved together with Z-L-Tyr(Bu^t)-Gly-Gly-OH (155 mg, 0.32 mmol) in *N*,*N*-dimethylformamide (DMF) (1.0 ml) and triethylamine (134 µl, 0.958 mmol). To this solution (cooled at 0 °C) dimethylphosphinothioic azide (47.6 mg, 0.352 mmol) in DMF (0.3 ml) was added and the mixture was stirred at this temperature for 24 h. The solution was diluted with ethyl acetate and washed with water, a 5% aq $NaHCO_3$ solution, water and saturated NaCl solution, dried over anhydrous sodium sulfate and evaporated. The oily residue was purified by silica-gel column chromatography to give **6** as amorphous solid; 207.8 mg (82%); $[\alpha]_D^{27}$ −13.4° (*c* 1.0, methanol); R_f^4=0.44, R_f^7=0.51. ^{1}H NMR ($CDCl_3$) δ=0.7—1.8 (m, 27H), 2.1—3.2 (m, 8H), 3.3—5.1 (m, 8H), 6.3—8.0 (m, 18H).

Found: C, 66.79; H, 7.76; N, 9.10%. Calcd for $C_{44}H_{61}O_8N_5$: C, 67.07; H, 7.80; N, 8.89%.

Preparation of H-L-Tyr-Gly-Gly-L-Pheψ[CH$_2$-NH]L-Leu-OH (7): 25% HBr in acetic acid (0.4 ml) was added to a solution of **6** (54.7 mg, 0.069 mmol) in acetic acid (0.2 ml) containing anisole (0.1 ml) and the solution stirred at room temperature for 2 h. After evaporation, the residue was washed with ether by trituration. The crude product was purified by silica-gel TLC by a solvent system of chloroform–methanol–29% aqueous ammonia (65:25:4) followed by gel chromatography on Sephadex LH-20 (eluted with methanol) to give chromatographically homogeneous monohydrobromide dihydrate of **7** as amorphous solid; 38.0 mg (83%); $[\alpha]_D^{27}$ +23.0° (*c* 1.9, methanol); R_f^5=0.25, R_f^8=0.54.

Found: C, 51.01; H, 6.42; N, 10.41%. Calcd for $C_{28}H_{42}O_7N_5Br$: C, 51.06; H, 6.73; N, 10.63%.

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