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Simple Peptides. II.[1,2] Syntheses and Properties of Taurine-Dipeptides Containing Neutral α-Amino Acid

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The physicochemical and immunochemical properties of fourteen taurine dipeptides (**1**—**14**) containing one of eleven neutral L-amino acids or three neutral D-amino acids were examined. These taurine peptides were synthesized by a conventional method using chemical coupling of taurine with an amino acid. Alternatively, the dipeptides (**1**—**9**) containing L-amino acid were also synthesized, with a few exceptions (**10**, **11**), by a new method using a general substitution reaction of a sulfo group for chlorine or bromine *via* the β-halogenoethyl amide of each amino acid. When sodium sulfite or ammonium sulfite was used in the substitution, the new method gave pure taurine dipeptides in good yield without racemization. All the dipeptides (**1**—**14**), including naturally occurring Ser–Tau, showed high cross-reactivities with antisera against haptenic taurine or γGlu–Tau.

Keywords——taurine; taurine-containing dipeptide; synaptic peptide; Ser–Tau; immuno-crossreactivity; anti-taurine serum; anti-γGlu–Tau serum; neuroscience

Taurine is the most abundant amino acid, being distributed widely in the body, and various biological activities of this sulfur-containing amino acid have been reported.[3] Unlike many other amino acids, taurine has been believed not to be incorporated in polypeptides or proteins because of its unique chemical structure. However, the recent discovery of γGlu–Tau (**10**: glutaurine) extracted from mammalian parathyroids[4] prompted further studies on the possible existence of taurine-oligopeptides in mammalian brain.[5-8] Studies on the identification and biological activity of such brain oligopeptides require the availability of various authentic taurine-peptides, but so far only glutaurine is well documented.[9,10] It was one of the aims of the present study to supply synthetic samples for such a purpose.

Another aim of this work was to examine the cross-immunoreactivity of taurine-peptides with an antibody against haptenic taurine. This is because the taurine antibody, when applied in immunohistochemistry,[11,12] is a very powerful tool for the microscopic visualization of cells containing "taurine-like immunoreactivity (TLI)." Thus, neuroanatomical mapping of TLI in the brain contributes to the understanding of the physiological roles of taurine. As the term implies, however, the TLI may include not only taurine but also other unknown compounds. This suspicion should be examined by testing the immunological cross-reactivity of taurine antibodies with various taurine-related materials. Although the taurine antibody we have produced previously little cross-reacted with many other amino acids,[11,12] it did cross-react with glutaurine and Gly–Tau almost as effectively as it reacted with taurine.[13] These facts indicate that the possible existence of taurine-peptides must be taken into consideration in the evaluation of immunohistochemical results using taurine antiserum; TLI observed in the brain may reflect the possible existence of some taurine oligopeptides. Evidently, the

H-X-Tau-OH

X: Gly (**1**), Ala (**2**), Val (**3**), Leu (**4**), Ile (**5**), Pro (**6**), Hyp (**7**), Ser (**8**), Thr (**9**), Phe (**10**), Tyr (**11**)

H-D-X-Tau-OH

X: Ala (**12**), Val (**13**), Ser (**14**)

Z-X-NH-CH_2CH_2-Cl

X: Gly (**15**), Ala (**16**)

Boc-X-NH-CH_2CH_2-Cl

X: Gly (**17**), Ala (**18**), Ser (**19**)

Boc-X-NH-CH_2CH_2-Br

X: Gly (**20**), Ala (**21**), Val (**22**), Leu (**23**), Ile (**24**), Pro (**25**), Hyp (**26**), Ser (**27**), Thr (**28**)

H-X-NH-CH_2CH_2-Cl

X: Gly (**29**), Ala (**30**), Ser (**31**)

H-X-NH-CH_2CH_2-Br

X: Gly (**32**), Ala (**33**), Val (**34**), Leu (**35**), Ile (**36**), Pro (**37**), Hyp (**38**), Ser (**39**), Thr (**40**)

H-Tau-OH: (**41**)

Chart 1

method A

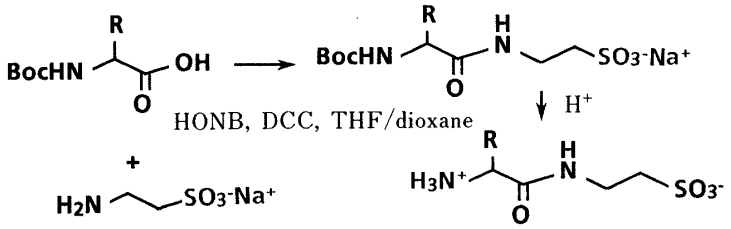

Chart 2

availability of a series of taurine peptides is of great importance for the determination of the immunorecognition site(s) of the taurine antibody.

Eleven taurine dipeptides (**1**—**11**) containing a neutral L-amino acid and three (**12**—**14**) containing a neutral D-amino acid were synthesized by the conventional method using chemical coupling as shown in Chart 2. Although some of these authentic dipeptides (**2**—**4**, **10**) have been described in a Japanese patent,[14] some of the reported physicochemical properties (such as melting point) differ considerably from our data. In the experimental section, therefore, we described in detail the method of synthesis we employed and the properties of materials we obtained.

In order to confirm the above data, we applied a new substitution method, rather than other known chemical[9,10,15] or enzymatic[16] coupling methods, for an alternative synthesis of taurine dipeptides and compared the results. The new method is characterized by the use of a general substitution reaction which provides taurine-dipeptides from corresponding amino acid β-halogenoethyl amides as illustrated in Chart 3 and Table I. The substitution of a sulfo group for chlorine or bromine was achieved by using sodium sulfite or ammonium sulfite. It

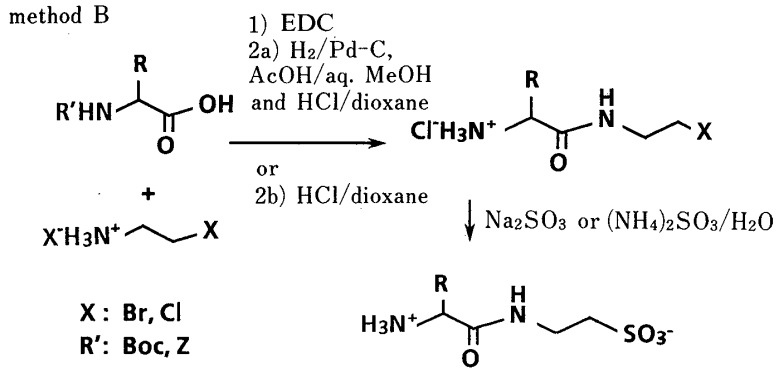

Chart 3

TABLE I. The Substitution of Halogenoethyl Amides to Obtain Taurine-Dipeptides

$$\text{H–A–NH–CH}_2\text{CH}_2\text{X}\cdot\text{HCl} \longrightarrow \text{H–A–NH–CH}_2\text{CH}_2\text{SO}_3\text{H}$$
(A–Tau)

Amides (A, X)	Time (h)	Temp. (°C)		Yield (%)	Products	mp (°C) (dec.)
29 (Gly, Cl)	24	50	Na	70	**1**	314—315
30 (Ala, Cl)	170	25	Na	44	**2**	314—315
31 (Ser, Cl)	170	25	Na	52	**8**	265—267
32 (Gly, Br)	24	25	Na	93	**1**	316—318
33 (Ala, Br)	48	25	Na	70	**2**	314—315
33 (Ala, Br)	48	25	NH_4	68	**2**	315—316
34 (Val, Br)	48	25	Na	78	**3**	313—315
34 (Val, Br)	48	25	NH_4	61	**3**	313—315
35 (Leu, Br)	48	25	Na	49	**4**	303—305
36 (Ile, Br)	48	25	Na	50	**5**	315—317
37 (Pro, Br)	48	25	Na	51	**6**	247—248
38 (Hyp, Br)	48	25	Na	53	**7**	280—282
39 (Ser, Br)	48	25	Na	68	**8**	266—267
40 (Thr, Br)	48	25	Na	45	**9**	254—256

was found that bromo derivatives were generally better intermediates than chloro derivatives, and that sodium sulfite and ammonium sulfite were equipotent. All neutral α-amino acids examined by this method were L-form (but D-form may be prepared similarly). Phe–Tau (**10**) and Tyr–Tau (**11**) could not be obtained in good yields because of unforeseen side reaction(s).

Since the substitution reaction required a long reaction time, proof of the optical purity of the product was thought to be very important, especially in the case of Ser–Tau (**8**), which is a naturally occurring dipeptide, because it contains Ser. All products (**1**—**9**) obtained by the new method had almost the same $[\alpha]_D$ values as those obtained by the conventional method. Comparison of the $[\alpha]_D$ values of three peptides (L-form, **2**, **3**, **8**; D-form, **12**—**14**) obtained by the two methods suggested that no racemization occurred in the new substitution method. Direct proof was obtained in the case of L-Val–Tau (**3**) by reverse-phase high performance liquid chromatography (HPLC) with a chiral mobile phase, Cu(L-Pro)$_2$, which can distinguish L- and D-isomers.[17] Figure 1 shows the L-form substitution product (**3**) with the contaminant D-isomer amounting to less than 0.1%.

It is interesting to note that $[\alpha]_D$ values of these taurine-dipeptides were similar to those of GABA-dipeptides[1] in which GABA is located at the C-terminal under acidic conditions. In both GABA- and taurine-dipeptides, except for the Thr, Phe and Tyr derivatives (**9**, **10**, **11**),

TABLE II. Specific Optical Rotation Values at the Sodium D Line of Taurine-Dipeptides and Their Amino Acid Components[a] at 20—25 °C

Compd.	Dipeptides (H–X–Y–OH) $[\alpha]_D^{20}$ ($c=1$, H_2O) Method A	Method B	Amino acid components (H–X–OH) $[\alpha]_D$ ($c=1$—5, H_2O)	$[\alpha]_D$ ($c=1$—5, aq. HCl)
2	+9.4°	+9.1°	+1.8°	+14.6°
12	−9.1°	—	−1.8°	−14.6°
3	+40.5°	+43.5°	+5.6°	+28.3°
13	−40.1°	—	−5.6°	−28.3°
4	+31.9°	+31.7°	−11.0°	+16.0°
5	+38.7°	+38.9°	+12.4°	+39.5°
6	−44.8°	−44.5°	−86.2°	−60.4°
7	−35.5°	−35.8°	−76.0°	−50.5°
8	+10.1°	+9.9°	−7.5°	+15.1°
14	−8.9°	—	+7.5°	−15.1°
9	+18.8°	+18.6°	−28.5°	−15.5°
10	+69.2°	—	−34.5°	−4.5°
11	+27.6° [b]	—	[c]	−10.5°

a) "Data for Biochemical Research," ed. by R. M. C. Dawson, D. Eliott, W. H. Eliott and K. M. Jones, Clarendon Press, Oxford 1974. *b*) $c=0.5$, 0.1 N NaOH. *c*) Insoluble.

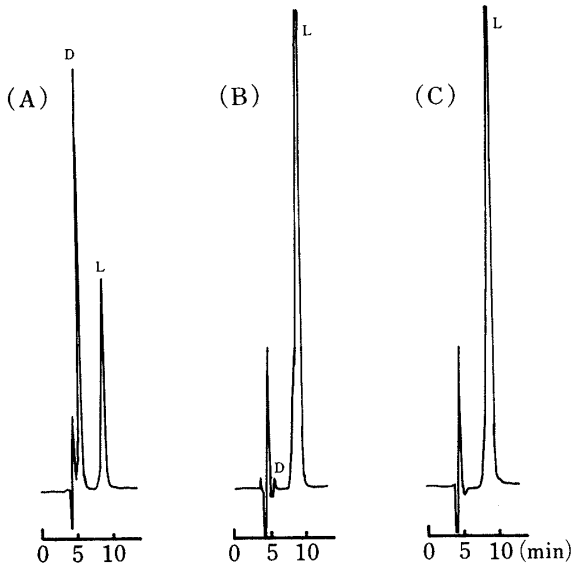

Fig. 1. HPLC Separation of D- and L-Val–Tau with Chiral Mobile Phase

(A) Equimolar mixture of D- and L-isomers. (B) L-Isomer with 1.0% D-isomer. (C) Val–Tau prepared by method B. HPLC conditions were as follows. Column: Develosil ODS-7 (4.6×250 mm). Eluent: 1 mM $Cu(OAc)_2$ (pH 6.2) containing 2.1 mM L-Pro. Flow rate: 0.5 ml/min. Temp.: 40 °C. Detect.: 205 nm (UV).

the $[\alpha]_D$ values seem to depend not on the total charge of the molecules, but on the charge of the hetero atom next to the carbonyl group.

Table III shows the δ and J values of dipeptides (**1**—**11**) in 0.1 N DCl, taken from the proton magnetic resonance spectra. The spectra of dipeptides containing D-amino acid (**12**—**14**) and the corresponding L-isomers (**2**, **3**, **8**) were identical. Signals derived from Tau were characteristic, and the chemical shifts of αH and βH of the Tau residue in these peptides were shifted slightly to the upper field from those of Tau itself. The peptides containing Val (**3**), Ile (**5**) or Thr (**9**), as well as Gly (**1**), gave one kind of βH proton signal, while the other peptides (**2**, **4**, **6**—**8**, **10**, **11**) had two kinds of βH signals. It should be noted that two different signals of αH proton were also observed in six dipeptides (**3**—**5**, **10**, **11**, **13**) among the fourteen.

The naturally occurring dipeptide Ser–Tau (**8**) was first synthesized, and studies on its distribution and biological roles in neural tissue should be facilitated thereby.

TABLE III. Proton Magnetic Resonance Data for Dipeptides (H–X–Tau–OH) with C-Terminal Taurine (H–Tau–OH)

	Signals of N-terminal moiety (–X–)	Signals of C-terminal moiety (–Tau–)
1	3.80 (2H, s)	3.10 (t, J=6.5, αH), 3.63 (t, J=6.5, βH)
2	1.50 (3H, d, J=7.5), 4.05 (1H, q, J=7.5)	3.10 (t, J=6.5, αH), 3.61, 3.63 (ddd, J=6.5, 6.5, 14, βH)
3	1.01 (3H, d, J=7), 1.02 (3H, d, J=7), 2.15—2.25 (1H, m), 3.77 (1H, d, J=7.5)	3.09, 3.13 (ddd, J=6.5, 6.5, 14, αH) 3.65 (t, J=6.5, βH)
4	0.93 (3H, d, J=6), 0.95 (3H, d, J=6), 1.61—1.78 (3H, m), 3.97 (1H, t, J=7.5)	3.08, 3.12 (ddd, J=6.5, 6.5, 14, αH), 3.61, 3.65 (ddd, J=6.5, 6.5, 14, βH)
5	0.92 (3H, t, J=7), 0.98 (3H, d, J=7), 1.14—1.30 (1H, m), 1.45—1.56 (1H, m), 1.90—2.01 (1H, m), 3.84 (1H, d, J=6)	3.08, 3.12 (ddd, J=6.5, 6.5, 14, αH), 3.64 (t, J=6.5, βH)
6	2.00—2.12 (3H, m), 2.35—2.46 (1H, m), 3.33—3.48 (2H, m), 4.32—4.38 (1H, m)	3.10 (t, J=6.5, αH), 3.61, 3.66 (ddd, J=6.5, 6.5, 14, βH)
7	2.18 (1H, ddd, J=4, 10, 14), 2.47 (1H, dddd, J=1.5, 1.5, 7, 14), 3.41 (1H, ddd, J=1.5, 1.5, 13), 3.50 (1H, dd, J=4, 13), 4.57 (1H, dd, J=7, 10), 4.70 (1H, dddd, J=1.5, 1.5, 4, 4)	3.10 (t, J=6.5, αH), 3.62, 3.67 (ddd, J=6.5, 6.5, 14, βH)
8	3.93 (1H, dd, J=6, 13), 3.98 (1H, dd, J=4, 13), 4.11 (1H, dd, J=4, 6)	3.11 (t, J=6.5, αH), 3.63, 3.66 (t, J=6.5, 6.5, 14, βH)
9	1.29 (3H, d, J=6.5), 3.85 (1H, d, J=6.5), 4.16 (1H, dq, J=6.5, 6.5)	3.12 (t, J=6.5, αH), 3.65 (t, J=6.5, βH)
10	3.15 (1H, dd, J=7.5, 14), 3.20 (1H, dd, J=7.5, 14), 4.17 (1H, t, J=7.5), 7.25—7.30 (2H, m), 7.34—7.44 (3H, m)	2.83, 2.93 (ddd, J=7, 7, 14, αH), 3.47, 3.53 (ddd, J=7, 7, 14, βH)
11	3.06 (1H, dd, J=7.5, 14), 3.12 (1H, dd, J=7.5, 14), 4.11 (1H, t, J=7.5), 6.88 (2H, d, J=8), 7.14 (2H, d, J=8)	2.86, 2.95 (ddd, J=7, 7, 14, αH), 3.47, 3.53 (ddd, J=7, 7, 14, βH)
41		3.24 (t, J=6.5, αH), 3.41 (t, J=6.5, βH)

δ values from external *tert*-BuOD (1.23), J in Hz, measured in 0.1 N DCl/D_2O.

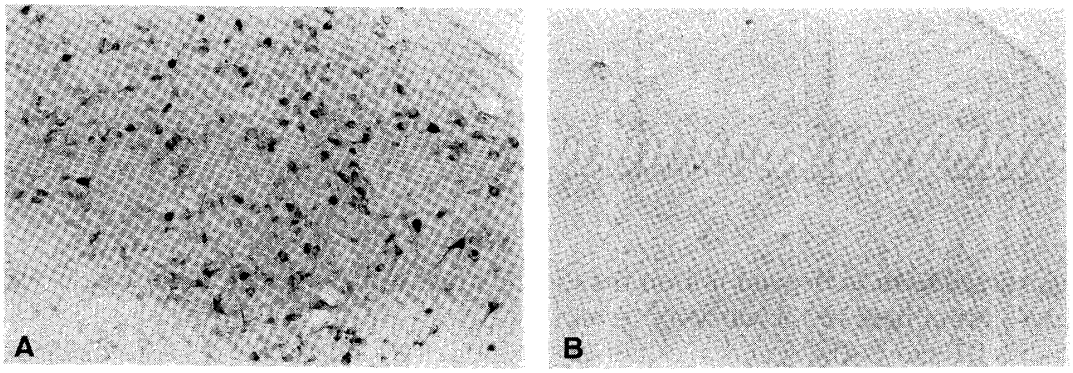

Fig. 2. Two Serial Sections of the Lateral Septum Obtained from a Rat

A: γGlu–Tau-like immunoreactive neurons are densely scattered in the gray matter of the lateral septum, whereas the white matter (below left) and the ependymal layer lack immunoreactivity. B: Immunohistochemical control, stained with anti-γGlu–Tau serum which had been preabsorbed with Gly–Tau. Blank areas in the above right corner are the lateral ventricle. (×80)

As shown in a previous report[13] and in Fig. 2, the rat brain contains γGlu–Tau-like immunoreactivity as well as TLI. It should be emphasized that γGlu–Tau-like immunoreactive material(s) is precisely localized in neural somata and their processes (Fig. 2-A). Such a

clear localization of the immunoreactivity strongly suggests that the immunoreactive peptide has a specific biological function in neural structures. Furthermore, in our preliminary study (data not shown), the absorption test[1] in γGlu–Tau immunohistochemistry, in which various taurine-dipeptides (**1**—**14**) synthesized here had been pre-added to the γGlu–Tau antiserum, indicated that the staining intensity of positive neurons with γGlu–Tau-like immunoreactivity was affected variously by different taurine-peptides. This immunohistochemical absorption experiment is very important for analyzing the immunoreactive substance(s) contained in these neurons.

Similarly, since all dipeptides (**1**—**14**) show more or less immuno-crossreactivity with anti-taurine serum in the absorption test, quantitative crossreactivity–structure analysis may also offer a clue in searching for novel naturally occurring bio-active taurine-peptide(s). Since a similar immunochemical phenomenon has been reported with antisera against GABA,[1] such an immunochemical study using antisera against haptenic amino acids should be done very carefully in combination with biochemical studies.

Experimental

Melting points of all compounds are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained with a Brucker AM-400 spectrometer by using *tert*-butanol (1.23 ppm) as an internal standard. Optical rotations were measured with a JASCO DIP-140 spectrometer.

Immunostaining Procedure——The avidin–biotin-complex (ABC) method[18] was used as an immunohistochemical procedure. Immunostaining for taurine-like or γGlu–Tau-like immunoreactivity was carried out in rat brain tissues according to the procedure described by Tomida and Kimura.[13] For absorption tests, only the first step of the staining procedure was modified. Namely, a 0.3% Triton-X solution (100 μl), in which a test sample (0.3 mg or none) was dissolved, was added to a diluted solution of anti-Tau serum (1 ml; 1 : 10000) or anti-γGlu–Tau serum (1 ml; 1 : 20000). The absorption ability of the sample was judged from the staining intensity compared with that of the non-absorbed control.

Synthetic Method A (a Conventional Method)

H–Ser–Tau–OH (**8**): DCC (6.81 g) was added to a solution of Boc–Ser–OH (6.6 g) and HONB (5.91 g) in THF–dioxane (75 ml–75 ml) at 0 °C, and the mixture was stirred for an additional 20 min at the same temperature, then for 40 min at room temperature. The resulting DCU was filtered off, and the filtrate was concentrated *in vacuo*. The oily residue was dissolved in dioxane (75 ml), and to this solution, taurine sodium salt (30 mmol) dissolved in water (50 ml) was added at room temperature. After stirring of the mixture for 8 h followed by evaporation of the organic solvent *in vacuo*, the residual aqueous solution was washed with ethyl acetate. The solution was applied to a column packed with IR-120B (H^+ form) resin. The water eluate was evaporated to dryness *in vacuo* to give a crystalline powder, which was recrystallized from ethanol–water to give pure H–Ser–Tau–OH (**8**) (5.30 g/90%), mp 265—267 °C (dec.). *Anal.* Calcd for $C_5H_{12}N_2O_5S$: C, 28.30; H, 5.70; N, 13.20. Found: C, 28.37; H, 5.91; N, 12.88.

Similarly, ten taurine-peptides (**1**—**7**, **9**—**11**) containing an L-amino acid were obtained: H–Gly–Tau–OH (**1**) (75%), mp 316—318 °C (dec.). *Anal.* Calcd for $C_4H_{10}N_2O_4S$: C, 26.38; H, 5.53; N, 15.38. Found: C, 26.56; H, 5.80; N, 15.05. H–Ala–Tau–OH (**2**) (90%), mp 314—316 °C (dec.) [lit.[14] mp > 300 °C (dec.) for $C_5H_{12}N_2O_4S$, $[\alpha]_D$ +9.6° (c = 20.6, H_2O)]. *Anal.* Calcd for $C_5H_{12}N_2O_4S$: C, 30.61; H, 6.16; N, 14.28. Found: C, 30.53; H, 6.44; N, 14.16. H–Val–Tau–OH (**3**) (89%), mp 313—315 °C (dec.) [lit.[14] mp 271—274 °C (dec.) for $C_7H_{16}N_2O_4S \cdot H_2O$, $[\alpha]_D^{23}$ +45.9° (c = 22.0, H_2O)]. *Anal.* Calcd for $C_7H_{16}N_2O_4S \cdot H_2O$: C, 34.70; H, 7.49; N, 11.56. Found: C, 34.60; H, 7.29; N, 11.41. H–Leu–Tau–OH (**4**) (67%), mp 300—302 °C (dec.) [lit.[14] mp 280—282 °C (dec.) for $C_8H_{18}N_2O_4S \cdot H_2O$ $[\alpha]_D^{22}$ +33.8° (c = 22.0, H_2O)]. *Anal.* Calcd for $C_8H_{18}N_2O_4S$: C, 40.32; H, 7.61; N, 11.76. Found: C, 40.36; H, 7.86; N, 11.79. H–Ile–Tau–OH (**5**) (69%), mp 315—317 °C (dec.). *Anal.* Calcd for $C_8H_{18}N_2O_4S \cdot 2/3H_2O$: C, 38.39; H, 7.78; N, 11.19. Found: C, 38.58; H, 8.12; N, 11.20. H–Pro–Tau–OH (**6**) (81%), mp 242—244 °C. *Anal.* Calcd for $C_7H_{14}N_2O_4S$: C, 37.83; H, 6.35; N, 12.60. Found: C, 37.51; H, 6.04; N, 12.37. H–Hyp–Tau–OH (**7**) (72%), mp 278—280 °C (dec.). *Anal.* Calcd for $C_7H_{14}N_2O_5S$: C, 35.29; H, 5.92; N, 11.76. Found: C, 35.18; H, 5.98; N, 11.74. H–Thr–Tau–OH (**9**) (96%), mp 254—256 °C (dec.). *Anal.* Calcd for $C_6H_{14}N_2O_5S \cdot 1/5EtOH$: C, 32.65; H, 6.51; N, 11.90. Found: C, 32.70; H, 6.63; N, 12.01. H–Phe–Tau–OH (**10**) (75%), mp 306—308 °C (dec.) [lit.[14] mp 266—268 °C (dec.) for $C_{11}H_{16}N_2O_4S$, $[\alpha]_D^{23}$ +69.2° (c = 1.03, H_2O)]. *Anal.* Calcd for $C_{11}H_{16}N_2O_4S \cdot 1/3H_2O$: C, 47.47; H, 6.04; N, 10.07. Found: C, 47.53; H, 6.06; N, 10.13. H–Tyr–Tau–OH (**11**) (40%), mp 280—281 °C (dec.). *Anal.* Calcd for $C_{11}H_{16}N_2O_5S \cdot H_2O$: C, 43.13; H, 5.92; N, 9.14. Found: C, 42.92; H, 6.08; N, 9.00.

Three peptides (**12**—**14**) containing a D-amino acid were similarly prepared: H–D-Ala–Tau–OH (**12**) (90%), mp 304—306 °C (dec.). *Anal.* Calcd for $C_5H_{12}N_2O_4S$: C, 30.61; H, 6.16; N, 14.28. Found: C, 30.39; H, 6.40; N, 14.13. H–D-Val–Tau–OH (**13**) (72%), (54%), mp 314—315 °C (dec.). *Anal.* Calcd for $C_7H_{16}N_2O_4S \cdot H_2O$: C, 34.70; H, 7.49; N,

11.56. Found: C, 34.64; H, 7.83; N, 11.53. H-D-Ser-Tau-OH (**14**) (71%), mp 268—269 °C (dec.). *Anal.* Calcd for $C_5H_{12}N_2O_5S$: C, 28.30; H, 5.70; N, 13.20. Found: C, 28.33; H, 5.95; N, 12.90.

Synthetic Method B (a New General Method)

B-1. Amide Formation——Z-Gly-NH-CH_2CH_2-Cl (**15**): EDC·HCl (6.32 g) was added to a solution of Z-Gly-OH (6.28 g), 1-amino-2-chloroethane hydrochloride (3.83 g) and triethylamine (3.34 g) in dichloromethane (100 ml) at 0 °C, and the mixture was stirred for 2 h at the same temperature, and for 20 h at room temperature. The solvent was then evaporated off *in vacuo*. After addition of water to the residue, the solution was extracted with ethyl acetate. Drying over sodium sulfate and evaporation *in vacuo* gave a crystalline residue, which was washed with ether and filtered off to yield thin-layer-chromatographically pure Z-Gly-NH-CH_2CH_2-Cl (**15**) (7.25 g/93%), mp 117—118 °C.

Similarly, four protected chloroethyl amides (**16**—**19**) and nine protected bromoethyl amides (**20**—**28**) were synthesized: Z-Ala-NH-CH_2CH_2-Cl (**16**) (80%), mp 121—123 °C, $[\alpha]_D^{20}$ −20.8° ($c=1$, $CHCl_3$). Boc-Gly-NH-CH_2CH_2-Cl (**17**) (96%), mp 71—73 °C. Boc-Ala-NH-CH_2CH_2-Cl (**18**) (90%), mp 104—105 °C, $[\alpha]_D^{20}$ −40.7° ($c=1$, $CHCl_3$). Boc-Ser-NH-CH_2CH_2-Cl (**19**) (82%), mp 119—120 °C, $[\alpha]_D^{20}$ −46.6° ($c=1$, $CHCl_3$). Boc-Gly-NH-CH_2CH_2-Br (**20**) (53%) (oil). Boc-Ala-NH-CH_2CH_2-Br (**21**) (61%), mp 86—88 °C, $[\alpha]_D^{20}$ −27.6° ($c=1$, $CHCl_3$). Boc-Val-NH-CH_2CH_2-Br (**22**) (64%), mp 116—118 °C, $[\alpha]_D^{20}$ −21.0° ($c=1$, $CHCl_3$). Boc-Leu-NH-CH_2CH_2-Br (**23**) (41%), mp 91—93 °C, $[\alpha]_D^{20}$ −38.4° ($c=1$, $CHCl_3$). Boc-Ile-NH-CH_2CH_2-Br (**24**) (66%), mp 124—126 °C, $[\alpha]_D^{20}$ −21.8° ($c=1$, $CHCl_3$). Boc-Pro-NH-CH_2CH_2-Br (**25**) (53%), mp 85—87 °C, $[\alpha]_D^{20}$ −84.5° ($c=1$, $CHCl_3$). Boc-Hyp-NH-CH_2CH_2-Br (**26**) (22%), mp 151—152 °C (dec.), $[\alpha]_D^{20}$ −71.8° ($c=1$, $CHCl_3$). Boc-Ser-NH-CH_2CH_2-Br (**27**) (43%), mp 104—106 °C, $[\alpha]_D^{20}$ −31.8° ($c=1$, $CHCl_3$). Boc-Thr-NH-CH_2CH_2-Br (**28**) (45%), mp 74—76 °C, $[\alpha]_D^{20}$ −41.3° ($c=1$, $CHCl_3$).

B-2. Deprotection——a) H-Gly-NH-CH_2CH_2-Cl·HCl (**29**): Hydrogenation of **15** (5.4 g) over a Pd catalyst in the usual manner in a mixed solvent of MeOH-AcOH-H_2O (50 ml-30 ml-5 ml), followed by removal of the catalyst and evaporation of the solvent, gave a residue. The residue was dissolved in a mixed solvent of EtOH and toluene (20 ml-30 ml), and the mixture was again evaporated to dryness to give an oily residue. The residue was dissolved in 4 N HCl in dioxane (10 ml), and evaporation of the solvent gave a crystalline solid, which was recrystallized from ethanol to give pure H-Gly-NH-CH_2CH_2-Cl·HCl (**29**) (3.2 g/93%), mp 168—169 °C.

Similarly, H-Ala-NH-CH_2CH_2-Cl·HCl (**30**) (80%), mp 184—186 °C, $[\alpha]_D^{20}$ +4.6° ($c=1$, H_2O), was obtained.

b) H-Ser-NH-CH_2CH_2-Cl·HCl (**31**): A solution of Boc-Ser-NH-CH_2CH_2-Cl (**19**) (3.5 g) in 4 N HCl in dioxane (65 ml) was stirred at room temperature for 4 h. The resulting colorless crystalline powder was collected by filtration after concentration and washed with ether to give pure H-Ser-NH-CH_2CH_2-Cl·HCl (**31**) (2.6 g/99%), mp 124—126 °C, $[\alpha]_D^{20}$ +7.6° ($c=1$, H_2O).

Similarly, two chloroethyl amides (**29**, **30**) and nine bromoethyl amides (**32**—**40**) were obtained: H-Gly-NH-CH_2CH_2-Cl·HCl (**29**) (98%), mp 168—169 °C. H-Ala-NH-CH_2CH_2-Cl·HCl (**30**) (99%), mp 184—186 °C. H-Gly-NH-CH_2CH_2-Br·HCl (**32**) (98%), mp 150—152 °C. H-Ala-NH-CH_2CH_2-Br·HCl (**33**) (99%), mp 158—159 °C, $[\alpha]_D^{20}$ +0.2° ($c=1$, H_2O). H-Val-NH-CH_2CH_2-Br·HCl (**34**) (96%), mp 105—106 °C, $[\alpha]_D^{20}$ +8.0° ($c=1$, H_2O). H-Leu-NH-CH_2CH_2-Br·HCl (**35**) (100%) (hygroscopic amorphous). H-Ile-NH-CH_2CH_2-Br·HCl (**36**) (100%) (hygroscopic amorphous). H-Pro-NH-CH_2CH_2-Br·HCl (**37**) (98%) (oil). H-Hyp-NH-CH_2CH_2-Br·HCl (**38**) (97%), mp 129—131 °C, $[\alpha]_D^{20}$ −36.9° ($c=1$, H_2O). H-Ser-NH-CH_2CH_2-Br·HCl (**39**) (96%), mp 89—91 °C, $[\alpha]_D^{20}$ +3.8° ($c=1$, H_2O). H-Thr-NH-CH_2CH_2-Br·HCl (**40**) (100%) (oil).

B-3. Substitution Reaction——H-Gly-Tau-OH (**1**): A solution of H-Gly-NH-CH_2CH_2-Br·HCl (**32**) (2.17 g) and sodium sulfite (2.52 g) in water was stirred for 24 h at room temperature. The reaction mixture was passed through an IR-120B column (H^+ form) and washed with excess water. The combined water solution was concentrated *in vacuo*. Upon addition of ethanol, a crystalline solid was formed to give pure H-Gly-Tau-OH (**1**) (1.32 g/93%), mp 316—318 °C (dec.). *Anal.* Calcd for $C_4H_{10}N_2O_4S$: C, 26.38; H, 5.53; N, 15.38. Found: C, 26.32; H, 5.74; N, 15.11.

Similarly, three peptides (**1**, **2**, **8**) and eight peptides (**2**—**9**) were obtained from the corresponding chloro- and bromo- derivatives (Table I). All the peptides had the expected values of elemental analyses corresponding to those of the same species found in the corresponding materials prepared by synthetic method A.

References and Notes

1) For Simple Peptides Part I, see: K. Ienaga, K. Higashiura and H. Kimura, *Chem. Pharm. Bull.*, **35**, 1249 (1987).
2) Amino acids, peptides and their derivatives, except Gly and Tau, are of the L-configuration, unless they are shown as D-form. Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). BOC = *tert*-butoxycarbonyl, DCC = *N*,*N*′-dicyclohexylcarbodiimide, DCU = *N*,*N*′-dicyclohexylurea, GABA = γ-aminobutyric acid, HONB = *N*-hydroxy-5-norbornene-2,3-dicarboximide, EDC = 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide, Z = benzyloxycarbonyl, THF = tetrahydrofuran.
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