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Simple Peptides. III.[1-3] Syntheses and Properties of Taurine-Oligopeptides Containing an Acidic α-Amino Acid

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Although taurine-dipeptides containing an acidic α-amino acid (Glu or Asp) have been proposed to exist in the central nervous system, it is not yet clear how the acidic amino acid is linked with taurine in the peptides. This study aimed to analyze the mode of linkage of such peptides through analysis of their spectral data, such as proton nuclear magnetic resonance and mass spectra. Four pairs of taurine-oligopeptides (**1**—**8**) containing one acidic α-amino acid were examined, and spattered ion mass spectrometry B/E linked scan mass spectrometry was proved to be most useful to distinguish each pair of isomers. Further, immunochemical examination indicated that all the oligopeptides (**1**—**8**) synthesized by the conventional coupling method were highly cross-reactive with antisera against taurine and against γGlu–Tau.

Keywords——taurine; γGlu–Tau (glutaurine); synaptic peptide; taurine-peptide; taurine-oligopeptide containing an acidic amino acid; SIMS B/E linked scan; mass spectrometry; neuroscience; immuno-crossreactivity; anti-taurine serum; anti-glutaurine serum

Although taurine itself, the most abundant amino acid in the human body, has been intensively investigated,[4] studies on taurine-peptides have been limited. Until 1980, no information had been available on the existence of naturally occurring taurine-peptides, probably because of the general concept that taurine might not be able to be incorporated into protein in the same way as other common α-amino acids. However, several papers have suggested that taurine-oligopeptides do exist in the mammalian brain since the discovery of γGlu–Tau (**2**, glutaurine) in mammalian parathyroids in 1980.[5] It was suggested that the formation of taurine-peptides occurred *in situ* without genetranscription. So far, no taurine-peptides containing basic amino acid(s) such as salty peptides[6] have been obtained from nervous tissue. However, a few presumed taurine-peptides have been found in neural tissue.[7-10] These peptides can be classified chemically into two groups; other than taurine, the first group contains only neutral amino acid(s), and the second group contains at least one acidic α-amino acid. In the case of the second group, however, it has not been determined, by using conventional techniques, how the acidic amino acid moiety is linked in such peptides, because of the difficulty in isolation of sufficient amounts of pure materials for structural analysis.

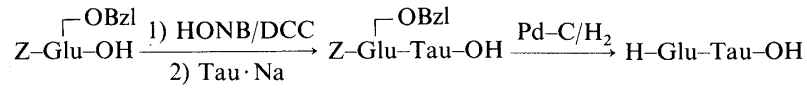
In our previous paper,[1] general syntheses and properties of the first group of taurine-dipeptides, including naturally occurring Ser–Tau, were reported. Here, we describe the synthesis of the second group of taurine-oligopeptides, in order to provide a basis for studying the distribution and function of the peptides in neural systems, and also for establishing a micro-analytical method, by which the mode of linkage of the acidic amino acid moiety can be distinguished.

H–X–Tau–OH
X: αGlu (**1**), γGlu (**2**), αAsp (**3**), βAsp (**4**)
H–X–Ser–Tau–OH
X: αGlu (**5**), γGlu (**6**)
H–Ser–X–Tau–OH
X: αGlu (**7**), γGlu (**8**)
H–Tau–OH: (**9**)

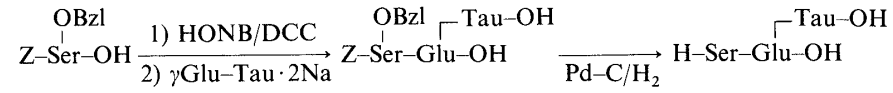
Chart 1

method A

a) αGlu–Tau



b) Ser–γGlu–Tau



method B

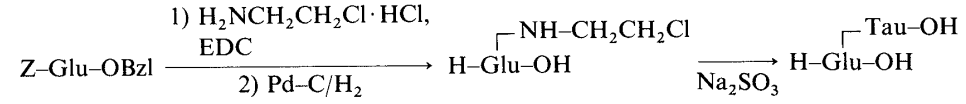


Chart 2

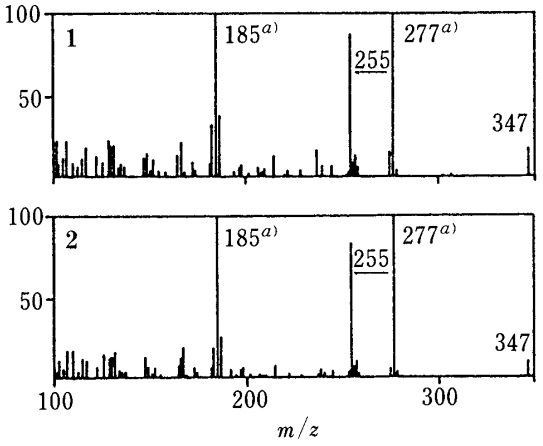


Fig. 1. SI Mass Spectra of L-Glu–Tau

The superscript *a*) indicates a peak due to background glycerol; the molecular weight of Glu–Tau is 254.

On the other hand, Kimura *et al.*[11-14] observed that antiserum against taurine had very high immuno-crossreactivity with naturally occurring glutaurine, even though an antiserum against glutaurine did not recognize taurine itself so well. Therefore, the synthesis of taurine-peptides (**1**—**8**) would also be valuable for a further systematic study on the immuno-recognition site.

Among several conventional synthetic methods, that require a chemical or enzymatic coupling step of two amino acid components,[6,15-17] the chemical active ester method, shown in Chart 2 and proved to be useful in the previous paper,[1] was employed as a general method. For comparison, glutaurine (**2**)[15,16] was also prepared by means of the substitution reaction *via* chloroethylamide as previously reported.[1] Peptides obtained by the two methods were identical.

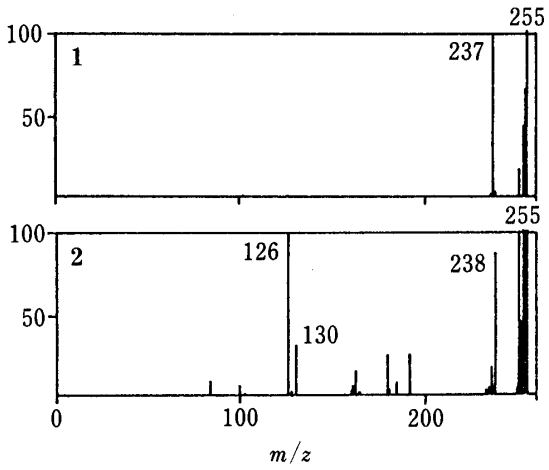


Fig. 2. SIMS B/E Linked Scan Mass Spectra of L-Glu–Tau

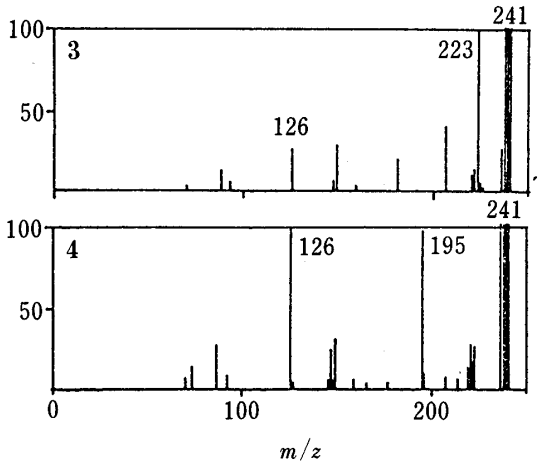


Fig. 3. SIMS B/E Linked Scan Mass Spectra of L-Asp–Tau

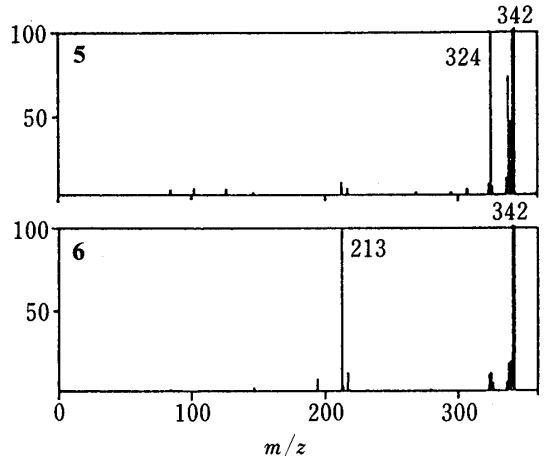


Fig. 4. SIMS B/E Linked Scan Mass Spectra of L-Glu–L-Ser–Tau

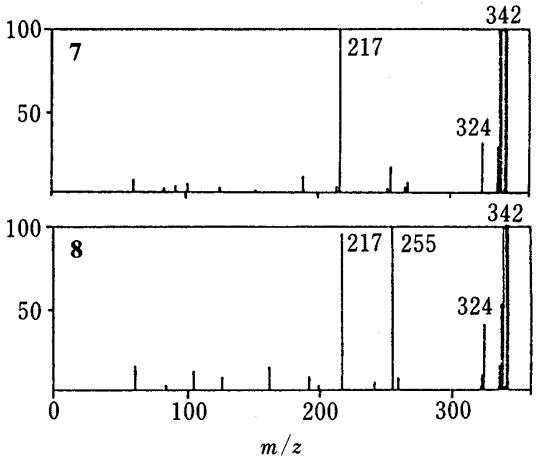


Fig. 5. SIMS B/E Linked Scan Mass Spectra of L-Ser–L-Glu–Tau

For differentiation of a pair of taurine-peptide isomers containing an acidic α-amino acid other than taurine, SIMS B/E linked scan mass spectrometry[18] was proved to be useful: the structures of all taurine-peptides may be identifiable if authentic samples are available; even without any authentic sample(s), the position of linkage of an acidic amino acid can be determined simply by analyzing its SIMS B/E linked scan mass spectrum (MS), if the acidic amino acid is located at the N-terminal and its amino group is free.

As shown in Fig. 1, αGlu–Tau (**1**) and γGlu–Tau (**2**) can not be distinguished by the usual SIMS technique. By contrast, in the SIMS B/E linked scan mass spectra (Fig. 2), each dipeptide gave a characteristic fragmentation pattern. Similarly, another pair of dipeptides [αAsp–Tau (**3**) and βAsp–Tau (**4**)] and a pair of tripeptides [αGlu–Ser–Tau (**5**) and γGlu–Ser–Tau (**6**)] were distinguishable by linked scan mass spectrometry as shown in Figs. 3 and 4. As a general rule, if the acidic amino acid is located at the N-terminal and its amino group is free, an α-linked isomer gives a characteristic $[MH-H_2O]^+$ peak, while an ω-linked Glu- or Asp-isomer gives a C-terminal ion, $[MH-129]^+$ or $[MH-115]^+$, respectively. As illustrated below, the latter two ions are apparently derived from the cleavage of the peptide bond. However, if the amino group of the acidic amino acid in question is blocked, the above general rule cannot be applied, though each pair of isomers is still distinguishable and

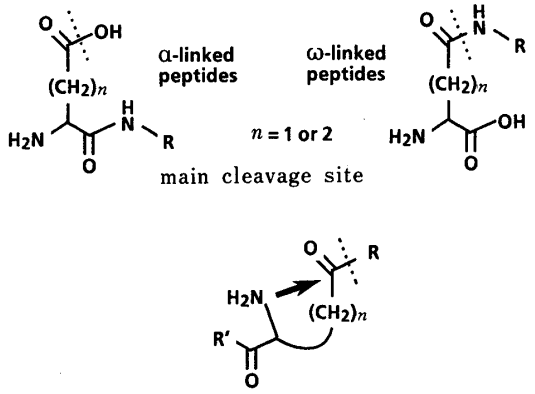


Chart 3

TABLE I. Proton Magnetic Resonance Data for Taurine-Oligopeptides

	Signals of amino acid(s) other than taurine	Signals of taurine moiety αH	βH
1	2.14 (1H, ddd, J=7, 7, 14), 2.19 (1H, ddd, J=7, 7, 14), 2.56 (2H, t, J=7), 4.03 (1H, t, J=7)	3.11 (t, J=6.5)	3.63 (t, J=6.5)
2	2.19 (1H, dddd, J=7.5, 7.5, 7.5, 15), 2.26 (1H, dddd, J=7.5, 7.5, 7.5, 15), 2.48 (1H, ddd, J=7.5, 7.5, 15), 2.52 (1H, ddd, J=7.5, 7.5, 15), 4.11 (1H, t, J=7.5)	3.08 (t, J=6.5)	3.57 (t, J=6.5)
3	3.00 (1H, dd, J=7.5, 18), 3.09 (1H, dd, J=5, 18), 4.34 (1H, dd, J=5, 7.5)	3.09 (t, J=7)	3.61, 3.66 (ddd, J=7, 7, 14)
4	2.99 (1H, dd, J=5, 17), 3.04 (1H, dd, J=6, 17), 4.38 (1H, dd, J=5, 6)	3.08 (t, J=6.5)	3.57, 3.61 (ddd, J=6.5, 6.5, 14)
5	2.20 (2H, dt, J=6.5, 7.5), 2.59 (2H, t, J=7.5), 3.83 (1H, dd, J=6, 12), 3.87 (1H, dd, J=5, 12), 4.16 (1H, t, J=6.5), 4.46 (1H, dd, J=5, 6)	3.09 (t, J=7)	3.58, 3.64 (ddd, J=7, 7, 14)
6	2.18—2.32 (2H, m), 2.60 (1H, dddd, J=7, 8, 16), 2.64 (1H, dddd, J=7, 8, 16), 3.83 (1H, dd, J=5, 11.5), 3.86 (1H, dd, J=5.5, 11.5), 4.13 (1H, t, J=7), 4.39 (1H, dd, J=5, 5.5)	3.06 (ttt, J=7, 7, 14), 3.09 (ttt, J=7, 7, 14)	3.57, 3.63 (ttt, J=7, 7, 14)
7	1.94—2.19 (2H, m), 2.42—2.55 (2H, m), 3.97 (1H, dd, J=5.5, 12), 4.01 (1H, dd, J=5, 12), 4.17 (1H, dd, J=5, 5.5), 4.39 (1H, dd, J=6, 8)	3.07 (t, J=7)	3.56, 3.61 (ddd, J=7, 7, 14)
8	1.98—2.29 (2H, m), 2.35—2.41 (2H, m), 3.96 (1H, dd, J=6, 12), 4.04 (1H, dd, J=4, 12), 4.19 (1H, dd, J=4, 6), 4.47 (1H, dd, J=5, 9)	3.07 (t, J=6.5)	3.50—3.64 (m)
9		3.24 (t, J=6.5)	3.41 (t, J=6.5)

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

identifiable by comparison with authentic samples. This fact also supports the idea that the neighboring effect, shown by an arrow in Chart 3, of a free α-amino group of an acidic amino acid is most important in the cleavage of the bond adjacent to the carboxy group in the ω-position of the same amino acid.

The linked scan mass spectra of a pair of tripeptides [Ser–αGlu–Tau (**7**) and Ser–γGlu–Tau (**8**)], which are isomers of **5** and **6**, are shown in Fig. 5. Evidently, blocking the amino group of the acidic amino acid did induce crucial changes in the linked scan mass spectra.

Differentiation of isomers by using the SIMS B/E linked scan mass spectra is much quicker and simpler than any classical chemical method, and the amount required for the

analysis is far less. For example, each pair of isomers can also be distinguished by proton nuclear magnetic resonance (^{1}H-NMR) as shown in Table I. However, this requires larger amounts of sample (more than ten times more) and a much longer time and more complicated procedures than those required in linked scan mass spectrometry. Moreover, without authentic samples, it seems potentially unreliable to determine the structures only by analysis of the ^{1}H-NMR spectra by using such a pH-dependent shift technique as described by Kasai and Sakamura.[19] This is because their general rule has not been applied well to the case of taurine dipeptides (**1**—**4**). Furthermore, the pH-controlled NMR measurement required in Kasai's method is not easy in our case because of the strong acidity of the taurine moiety. As compared with other techniques using mass spectrometry, the B/E linked scan technique has several advantages; it can be done by using the same sample after determination of its molecular weight by SIMS measurement, and it gives a simple pattern of peaks excluding those derived from the matrix.

This technique has therefore been applied as a general method to analyze the mode of linkage of oligopeptides containing acidic α-amino acid(s). The B/E linked scan technique in mass spectrometry is thought to be especially valuable to determine the structures of not only new taurine-peptide(s)[13] but also some known peptides[7,8] whose structures have not yet been determined.

All the peptides (**1**—**8**) were highly immuno-crossreactive, by judging the absorption test,[1,20] not only with anti-sera to taurine but also with anti-sera to glutaurine. Systematic studies on immuno-crossreactivity by using taurine-oligopeptides containing acidic and/or neutral amino acid(s) other than taurine are in progress.[14] Since a similar immuno-crossreactivity has been seen between GABA-peptides and anti-GABA serum,[20] the specificity of each antiserum against the haptenic-amino acid should be carefully examined.

Experimental

Melting points of all compounds are uncorrected. ^{1}H-NMR spectra were obtained by using *tert*-butanol (1.23 ppm) as an internal standard with a Bruker AM-400 spectrometer. Optical rotations were measured with a JASCO DIP-140 spectrometer. The SIMS spectra were obtained through the use of a Hitachi M-80B double focusing mass spectrometer with its standard SIMS source.

Mass Spectrometry——The mass scale was calibrated by using PFK with electron impact ionization. The primary ions were Xe^+, and the accelerating voltages of primary and secondary ions were 8 and 3 kV, respectively. Structurally informative fragment ions were obtained by a linked-scan (B/E) technique using $(M+H)^+$ ion as a precursor ion. Peptide samples were dissolved in water at a concentration of 2 μg/μl. Each sample solution (1.0—2.0 μl) was loaded on a stainless steel substrate, and about 0.5 μl of glycerol was layered over the sample on the substrate.

Method A——a) αGlu–Tau (**1**): DCC (4.54 g) was added to a solution of Z–Glu(OBzl)–OH·DCHA (11.06 g), *p*-TosOH·H_2O (3.80 g) and HONB (3.94 g) in dioxane–THF (50 ml/50 ml) at 0 °C, and the mixture was stirred at the same temperature for 2 h, then at room temperature for 20 h. The resulting DCUrea and *p*-TosOH·DCHA were filtered off, and the filtrate was concentrated *in vacuo*. The oily residue was dissolved in dioxane (50 ml), and then an aqueous solution of H–Tau–ONa, prepared from taurine (2.5 g) and $NaHCO_3$ (1.68 g) in H_2O (50 ml), was added at room temperature. After stirring of the reaction mixture for 20 h followed by evaporation of the organic solvent *in vacuo*, the remaining aqueous solution was washed with ethyl acetate. The solvent was evaporated off, and Z–Glu(Bzl)–Tau–ONa was obtained. Hydrogenation of the intermediate over a Pd catalyst in a mixed solvent of AcOH–MeOH–H_2O (40 ml–40 ml–20 ml), followed by removal of the catalyst and evaporation of the solvent, gave an oil. The residue was dissolved in water and applied to a column packed with IR-120B (H^+ form). After water elution and evaporation, the obtained oil was dissolved again in water and applied to a Dowex 1 (AcO^- form) column. The elute with 2 N AcOH, after adequate washing of the column with water, was evaporated to give a solid, which was recrystallized from water–propanol to yield pure αGlu–Tau (**1**), (3.47 g, 66%), mp 162—164 °C (dec.), $[\alpha]_D^{20}$ +40.6° (c=1.0, H_2O). *Anal.* Calcd for $C_7H_{14}N_2O_6S \cdot 0.5H_2O$: C, 31.94; H, 5.74; N, 10.64. Found: C, 31.92; H, 5.84; N, 10.54.

Three dipeptides (**2**—**4**) were similarly prepared from the corresponding Z–Glu(OH)–OBzl, Z–Asp(OBz)–OH and Z–Asp(OH)–OBzl. γGlu–Tau (**2**): (68%), mp 224—225 °C (dec.), $[\alpha]_D^{20}$ +20.3° (c=1.0, H_2O). *Anal.* Calcd for $C_7H_{14}N_2O_6S$: C, 33.07; H, 5.55; N, 11.02. Found: C, 33.23; H, 5.70; N, 11.15 [lit., 222—223 °C $[\alpha]_D^{28}$ +20.2° (c=3.61, H_2O)].[15] αAsp–Tau (**3**): (82%), mp 187—188 °C (dec.), $[\alpha]_D^{20}$ 17.1° (c=1.0, H_2O). *Anal.* Calcd for $C_6H_{12}N_2O_6S$: C,

30.00; H, 5.03; N, 11.66. Found: C, 29.72; H, 5.29; N, 11.41. βAsp–Tau (**4**): (74%), mp 80—110 °C (amorphous), $[\alpha]_D^{20}$ +4.4° (c=1.0, H_2O). *Anal.* Calcd for $C_6H_{12}N_2O_6S \cdot 0.2PrOH$: C, 31.43; H, 5.43; N, 11.11. Found: C, 31.41; H, 5.60; N, 10.85.

Two tripeptides (**5**, **6**) were similarly prepared by coupling of Z–Glu(OH)–OBzl or Z–Glu(OBzl)–OH with Ser–Tau. αGlu–Ser–Tau (**5**): (66%), mp 184—188 °C (amorphous), $[\alpha]_D^{20}$ +0.7° (c=1.0, H_2O). *Anal.* Calcd for $C_{10}H_{19}N_3O_8S \cdot 0.7H_2O$: C, 33.93; H, 5.81; N, 11.87. Found: C, 34.12; H, 5.85; N, 11.68. γGlu–Ser–Tau (**6**): (78%), mp 82—90 °C (amorphous), $[\alpha]_D^{20}$ −15.5° (c=1.0, H_2O). *Anal.* Calcd for $C_{10}H_{19}N_3O_8S \cdot 0.4H_2O \cdot 0.4PrOH$: C, 36.11; H, 6.22; N, 11.28. Found: C, 36.31; H, 6.34; N, 11.11.

b) Ser–γGlu–Tau (**8**): Z–Ser(Bzl)–ONB, prepared from Z–Ser(Bzl)–OH (3.29 g) by the same method as mentioned above, was dissolved in dioxane (40 ml), and then an aqueous solution of H–γGlu–Tau–ONa, prepared from H–γGlu–Tau–OH (2.54 g) and $NaHCO_3$ (1.68 g) in H_2O (40 ml), was added at room temperature. After stirring of the reaction mixture for 20 h followed by evaporation of the organic solvent *in vacuo*, the remaining aqueous solution was washed with ethyl acetate. The solvent was evaporated off, and Z–Ser(Bzl)–γGlu–Tau–ONa was obtained as a residue. Hydrogenation of the residue over a Pd catalyst, followed by the usual work-up, gave a solid, which was crystallized from water–propanol to yeild high-performance liquid chromatographically pure Ser–γGlu–Tau (**8**), (1.50 g, 44%), mp 184—187 °C (amorphous), $[\alpha]_D^{20}$ +6.0° (c=1.0, H_2O). *Anal.* Calcd for $C_{10}H_{19}N_3O_8S$: C, 35.19; H, 5.61; N, 12.31. Found: C, 35.03; H, 5.51; N, 12.02.

The α-linked isomer (**7**) was similarly prepared from H–αGlu–Tau–OH. Ser–αGlu–Tau (**7**), (40%), mp 211—213 °C (amorphous), $[\alpha]_D^{20}$ −16.4° (c=1.0, H_2O). *Anal.* Calcd for $C_{10}H_{19}N_3O_8S \cdot 0.1PrOH$: C, 35.62; H, 5.75; N, 12.10. Found: C, 35.66; H, 5.80; N, 11.88.

Method B——γGlu–Tau (**2**): γ-Glutamyltaurine (**2**) was synthesized by means of the substitution reaction reported previously as a general method:[1] the amide, Z–Glu($NHCH_2CH_2Cl$)–OBzl, obtained from Z–Glu(OH)–OBzl, was hydrogenated to give H–Glu($NHCH_2CH_2Cl$)–OH. This (30 mmol) was stirred with sodium sulfite (33 mmol) in water (60 ml) at room temperature for 2 d, and ion exchange chromatography gave pure crystalline γGlu–Tau (**2**), mp 223—224 °C [net yield from Z–Glu(OH)–OBzl: 20%].

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3) Amino acids, peptides and their derivatives, except Tau, are of the L-configuration. 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). GABA=γ-aminobutyric acid, Bzl=benzyl, Z=benzyloxycarbonyl, DCC=*N*,*N*′-dicyclohexylcarbodiimide, DCHA=dicyclohexylamine, DCUrea=*N*,*N*′-dicyclohexylurea, HONB=*N*-hydroxy-5-norbornene-2,3-dicarboxidime, THF=tetrahydrofuran, *p*-TosOH=*p*-toluenesulfonic acid, SIMS=spattered ion mass spectrometry.
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