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Studies on Peptides. LXXIX.[1,2] By-Products derived from N^{α}-Protected Tryptophan by Acids

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Four by-products formed during the treatment of Z(OMe)–Trp–OH with trifluoroacetic acid were isolated and their structures were assigned by nuclear magnetic resonance and mass spectra. It was found that the major by-product was H–Trp(2′-*p*-methoxybenzyl)–OH. Confirmatively, H–Trp(1′-*tert*-butyl)–OH was identified as the major by-product from Boc–Trp–OH. Such side reaction was found to be efficiently suppressed by the use of the scavenger system, such as, thioanisole containing 2% ethanedithiol–skatole or dimethylsulfide containing 2% ethanedithiol–skatole, during the deprotection with trifluoroacetic acid or preferably with 4 N ethanesulfonic acid.

Keywords——by-products derived from Boc–Trp–OH; by-products derived from Z(OMe)–Trp–OH; thioanisole (2% ethanedithiol)–skatole as scavengers; dimethylsulfide (2% ethanedithiol)–skatole as scavengers; H–Trp(1′-*tert*-butyl)–OH as a major by-product; H–Trp(2′-*p*-methoxybenzyl)–OH as a major by-product; 4 M ethanesulfonic acid as a deprotecting reagent

It has been known that the indole moiety of tryptophan is sensitive to oxidation under acidic conditions to produce pink colored by-products, however the formation of these could be suppressed by reducing reagents, such as mercaptoethanol[4] or ethanedithiol (EDT).[5] Regarding to the acid sensitivity of tryptophan, previously Uphaus *et al.*[6] pointed out the conversion of N-acetyl compounds to carboline derivatives and Theodorpoulos and Fruton[7] discussed the formation of β-oxindolylalanine. Recently Shimonishi *et al.*[8] reported the isolation of dimerized tryptophan derivative, as one of products derived from Ac-Trp-OMe under treatment with hydrogen fluoride or trifluoroacetic acid (TFA).

Despite of the presence of such a reducing reagent, when an acid labile protecting group, such as the Boc or Z(OMe) group, was removed from N^{α}-protected tryptophan by the usual treatment with TFA or dilute hydrochloric acid in the presence of a cation scavenger, such as anisole, a number of ninhydrin positive substances, besides the parent amino acid, could still be detected on thin-layer chromatography (TLC), though no colored substances were observed. Concerning to such side reactions, the partial *tert*-butylation of the tryptophan indole ring was first pointed out by Alakhov *et al.*,[9] after mass spectrometric examination of tryptophan peptides, derived from the TFA removal of the Boc or Bu^t ester group. Recently, Wünsch *et al.*[10] identified, among other products, two side products, H-Trp(1′-Bu^t)-OH and H-Trp(2′,5′,7′-tri-Bu^t)-OH, formed after the treatment of Boc-Trp-OH with TFA. The above

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[1] Part LXXVIII: H. Ogawa, M. Sugiura, H. Yajima, H. Sakurai, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **26**, 1549 (1978).

[2] Tryptophan is of the L-configuration. Following abbreviations were used: Boc=*tert*-butoxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bu^t=*tert*-butyl, Ac=acetyl, MBzl=*p*-methoxybenzyl.

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[8] Y. Omori, Y. Matsuda, S. Aimoto, Y. Shimonishi, and M. Yamamoto, *Chem. Lett.*, **1976**, 805.

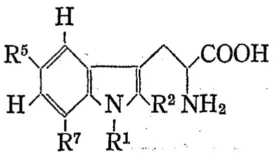
[9] Y.B. Alakhov, A.A. Kiryushkin, V.M. Lipkin, and G.W.A. Milne, *J. Chem. Soc. Chem. Commun.*, **1970**, 406.

[10] E. Wünsch, E. Jaeger, L. Kisfaludy, and M. Löw, *Angew. Chem.*, **89**, 330 (1977).

finding indicated that the indole moiety of tryptophan does act indeed as a scavenger of the *tert*-butyl cation liberated by acid like anisole and suggested that the cation trapping ability of anisole is not efficiently enough for the preparation of homogeneous peptides containing tryptophan by the acidolytic α-deprotecting procedure. More recently, Chino *et al.*[11] confirmed the 1′-substituted by-product formation and recommended the use of dimethylsulfide as a cation scavenger for suppression of this side reaction and Suzuki *et al.*[12] recommended the use of *p*-toluenesulfonic acid as a deprotecting reagent of the Boc group.

These situations prompted us to investigate the chemical nature observable during the treatment of Z(OMe)-Trp-OH with TFA in the presence of anisole (2 equiv. approximately 1/5 of the usual amount) containing 2% EDT. When examined by TLC, at least four components, besides the parent amino acid, were detected, though no pink color was observed. These products (M_1, M_2, M_3 and M_4 in the order of lower to higher *Rf* values) were separated by column chromatography on silica. The structure of these compounds were assigned as H-Trp(1′,5′-di-MBzl)-OH (M_1), H-Trp(2′-MBzl)-OH (M_2), H-Trp(2′,5′-di-MBzl)-OH (M_3) and H-Trp(2′,5′,7′-tri-MBzl)-OH (M_4) respectively as shown in Fig. 1 from infrared (IR), ^1H nuclear magnetic resonance (NMR) and mass spectral data. Especially the parent peak and the decarboxylation pattern in the mass spectra, the indole NH absorption band in IR and the particular proton signals (1′-H, 2′-H and 4′-H) in NMR spectra offered important clues to assign these formula for each compound. Only the structure of M_4 could not be fully characterized, because it occurred as a very minor component.

Fig. 1. Assigned Formula of By-products derived from Z(OMe)-Trp-OH and Boc-Trp-OH by Trifluoroacetic Acid



	R^1	R^2	R^5	R^7
M_1	MBzl	H	MBzl	H
M_2	H	MBzl	H	H
M_3	H	MBzl	MBzl	H
M_4	H	MBzl	MBzl	MBzl
B_1[a]	H	H	Bu^t	H
B_2	Bu^t	H	H	H
B_3	Bu^t	H	Bu^t	H
B_4	H	Bu^t	Bu^t	Bu^t

a) B_1 seems to be a mixture of three monosubstituted derivatives at 2′, 5′ and 7′.

Confirmatively, Boc-Trp-OH was similarly treated with TFA in the presence of anisole containing EDT. No pink color was observed, but four by-products, besides tryptophan, were detected on TLC. These compounds (B_1, B_2, B_3 and B_4 in the order of lower to higher *Rf* values) were similarly isolated by column chromatography on silica. Among these, two by-products were assigned as H-Trp(1′-Bu^t)-OH (B_2)[10,11] and H-Trp(1′,5′-di-Bu^t)-OH (B_3) respectively. The other two compounds (B_1 and B_4) could not be fully characterized. However, B_1 was postulated as a mixture of three mono-substituted derivatives, probably at 2′ or 5′ or 7′ position, since it gave the parent peak (M^+) at 260 in its mass spectrum and

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11) N. Chino, Y. Masui, and S. Sakakibara, "*Peptide Chemistry* 1977," ed. by T. Shiba, Protein Research Foundation, 1977, p. 27.

12) K. Suzuki, K. Nitsuta, Y. Endo, and A. Sasaki, *Seikagaku*, **48**, 424 (1976).

exhibited signals of three *tert*-butyl protons in NMR spectra. B_4, a very minor component, was concluded as the tri-substituted compound from its mass spectral data and therefore, as the most plausible formula, H-Trp(2′,5′,7′-tri-Bu^t)-OH[10] was tentatively assigned.

In these parallel experiments, M_2 and B_2 were found to occur as the major by-products respectively. These results indicated that the Bu^t cation has a great tendency to attack the nitrogen atom of indole predominantly and the MBzl cation at the 2′ position. Indeed, H-Trp(1′-MBzl)-OH, comparable to H-Trp(1′-Bu^t)-OH (B_2), was not found in the case of Z(OMe)-Trp-OH.

Chemical behaviour of Z(OMe)-Trp-OH and Boc-Trp-OH under the system of TFA-anisole containing EDT was thus mostly clarified, though amounts of by-products were different from each other, as will be mentioned later, depending on presumably the life time and electrophilic and steric properties of these cations to anisole or tryptophan. These results suggested in turn that indole derivatives, such as skatole, may play a role as a candidate of scavengers at the acidolytic deprotection step. When a mixture of Z(OMe)-Gly-OH and skatole was treated with TFA, at least two products presumably derived from intermolecular alkylation reaction of the MBzl cation were detected on TLC.

Next, using the Shimadzu dual wavelength TLC scanner, amounts of by-products derived from Boc-Trp-OH and Z(OMe)-Trp-OH in the presence of various scavengers, including skatole, were estimated and the results were listed in Table I and II respectively. Since ninhydrin color intensity of these products may differ from each other, very accurate comparison is not possible by this method. However, certain tendencies can be observed.

When Boc-Trp-OH was treated with TFA (Table I), among scavengers tested, thioether compounds, such as thioanisole or especially dimethylsulfide recommended by Chino *et al.*,[11]

TABLE I. By-products derived from Boc-Trp-OH by Acids

Acid	Scavenger	Adduct		Trp	B_1	B_2	B_3
		2% EDT	Skatole				
TFA	Anisole	—	—	59.4	18.9	21.9	Trace
		+	—	72.0	14.0	14.0	
		+	2 eq.	80.0	6.4	13.5	
		+	10 eq.	80.8	5.3	14.0	
	o-Cresole	—	—	51.2	18.0	30.7	Trace
		+	—	61.6	15.8	22.5	
	Thioanisole	—	—	67.6	9.9	22.5	
		+	—	73.6	9.7	16.7	
		+	2 eq.	80.7	5.3	18.2	
		+	10 eq.	83.5	5.0	11.5	
	Dimethylsulfide	—	—	80.4		19.6	
		+	—	81.5		18.5	
		+	2 eq.	81.7		18.3	
4N ESA	Anisole	—	—	66.1	7.4	26.5	Trace
		+	—	77.3		22.7	
		+	2 eq.	79.9		20.1	
		+	10 eq.	80.2		19.8	
	Thioanisole	—	—	83.9	2.7	13.5	
		+	—	96.6		3.4	
		+	2 eq.	96.8		3.2	
		+	10 eq.	97.5		2.5	
	Dimethylsulfide	—	—	87.2		12.8	
		+	—	87.7		12.3	
		+	2 eq.	97.3		2.7	
		+	10 eq.	99.0		1.0	
			Rf_2	0.56	0.66	0.68	0.75

Table II. By-products derived from Z(OMe)-Trp-OH by Acids

Acid	Scavenger	Adduct		Trp	M_1	M_2	M_3
		2% EDT	Skatole				
TFA	Anisole	–	—	32.5	30.0	33.8	3.8
		+	—	51.2	17.3	31.5	
		+	2 eq.	61.5	17.6	20.9	
		+	10 eq.	69.6	17.4	13.0	
	o-Cresole	–	—	38.8	23.5	31.7	6.0
		+	—	42.2	26.5	26.5	4.8
	Thioanisole	–	—	71.0	7.0	22.0	
		+	—	76.4	11.8	11.8	
		+	2 eq.	76.6	7.8	15.6	
		+	10 eq.	82.6	4.2	13.2	
	Dimethylsulfide	–	—	80.8	2.1	17.1	
		+	—	82.7	2.0	15.3	
		+	2 eq.	83.0	1.5	15.5	
4 N ESA	Anisole	–	—	48.3	17.4	32.0	2.3
		+	—	59.1	20.0	21.0	
		+	2 eq.	60.2	13.6	26.1	
		+	10 eq.	70.6	12.1	17.3	
	Thioanisole	–	—	52.6	7.2	37.3	2.9
		+	—	56.1	2.8	38.3	2.8
		+	2 eq.	66.0	7.0	27.0	
		+	10 eq.	82.4	3.0	15.6	
	Dimethyl-sulfide	–	—	67.1	9.2	23.7	
		+	—	78.1		21.9	
		+	2 eq.	85.9	5.1	9.0	
		+	10 eq.	90.5	3.8	5.7	
			Rf_2	0.56	0.64	0.70	0.77

seem to be much better scavengers than anisole or *o*-cresole. A thiol compound, EDT, suppressed significantly the pink color formation and in a certain degree, the by-product formation also. Some amount of skatole improved also these situations in a certain degree. In 1977, we reported that dilute ethanesulfonic acid, rather than *p*-toluenesulfonic acid, is a preferable deprotecting reagent for the Boc and the Z(OMe) groups.[13] When 4 N ethanesulfonic acid (ESA) in trifluoroethanol (TFE)[14] was employed as a deprotecting reagent, the recovery of tryptophan became somewhat better than that observed in the TFA system. Especially, in the system of thioanisole containing 2% EDT–skatole or dimethylsulfide containing 2% EDT–skatole, the tryptophan recovery of around 97—99% could be expected.

When Z(OMe)-Trp-OH was treated with TFA, total amounts of the by-products seemed to be greater than those derived from Boc-Trp-OH, presumably due to the comparable stability of the MBzl cation liberated by acid. At the best, when 4 N ESA was employed, the tryptophan recovery of 90% was obtained in the system of dimethylsulfide containing 2% EDT–skatole. In the system of thioanisole containing 2% EDT–skatole, the recovery was 82%.

These results indicated that unless otherwise finding a scavenger which has far efficient property than that of the indole of tryptophan, complete suppression of the alkylation seems difficult to establish and at present, we have to expect the maximal suppression by summing properties of available scavengers, such as thioanisole or dimethylsulfide plus skatole and EDT, as we examined above. Relatively increased amounts of scavengers may act efficiently

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[14] B. Riniker, B. Kamber, and P. Sieber, *Helv. Chim. Acta*, **58**, 1086 (1975).

to suppress this type of side reaction. The deprotecting reagent, 4 N ESA, seems better than TFA. However, the solvent system of ESA has to be changed, for example, from acetic acid to TFE or others, depending on the solubility of protected peptides, as we demonstrated in the synthesis of duck glucagon.[15] The side reaction was detectable most seriously, when Boc-Trp-OH or Z(OMe)-Trp-OH was treated with acids and seemed to become progressively less from relatively large peptides, as we encountered during the above glucagon synthesis. This seems due to decrease of molar concentration of the cation liberated from these peptides according to the increase of their molecular weight.

The data presented here indicated that for the synthesis of tryptophan peptides, the Boc group seems to be a preferable protecting group compared to the Z(OMe) group and may offer useful information for the synthesis of more homogeneous peptides containing tryptophan by the acidolytic α-deprotecting procedure. Recently, Kenner *et al.*[16] and Ueki and Ikeda[17] introduced the new protecting groups, diphenylphosphinic (Dpp) and diphenylphosphinothioyl (Ppt) groups respectively. The protecting groups which generate no cation at the deprotecting step seem to be the attractive ones for this purpose.

Experimental

Thin-layer chromatography was performed on silica (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8:3:1), Rf_2 *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2). ^1H-NMR spectra were determined by the Varian A-60 and Varian HA-100 spectrometer and mass spectra were taken with JMS-01SG-2 spectrometer (Japan Electron Optics) by a direct heated inlet system.

Isolation of the By-products derived from Z(OMe)-Trp-OH——Z(OMe)–Trp–OH (3.68 g, 10 mmol) was treated with TFA (5 ml) in the presence of anisole (2.2 ml, 2 equiv. approximately 1/5 of the usual amount) containing 2% EDT in an ice-bath for 60 min (three spots, M_1, M_2 and M_3, besides Trp, were detected on TLC) and the excess TFA was removed by evaporation *in vacuo* at 25° (while a new faint spot, M_4, appeared). The residue was washed with *n*-hexane several times and dissolved in AcOEt. The organic phase was washed five times with H_2O to remove tryptophan, dried over Na_2SO_4 and then evaporated. The residue was dissolved in a small amount of the solvent consisting of $CHCl_3$–MeOH–H_2O (8:3:1) and the solution was applied to a column of silica (2.0×37.5 cm, Kiesel Gel 60), which was eluted with the same solvent system. By repeated chromatography, four components with different *Rf* values were isolated; M_1 18 mg (Rf_1 0.20), M_2 68 mg (Rf_1 0.35), M_3 21 mg (Rf_1 0.47) and M_4 5 mg (Rf_1 0.49).

H–Trp(1′,5′-di-MBzl)–OH (M_1): mp 185—188° (recrystallized from MeOH and AcOEt). IR (KBr): No indole NH band at 3380 cm^{-1}. MS *m/e*: 444 (M^+ $C_{27}H_{28}N_2O_4$), 370 (444$-C_2H_4NO_2$), 250 (370$-C_8H_8O$), 121 (MBzl). NMR (DMSO-d_6) δ: 3.70 (6H, s, 2×OMe), 7.14 (1H, s, 2′-H), 7.40 (1H, broad s, 4′-H).

H–Trp(2′-MBzl)–OH (M_2): mp 189—190° (recrystallized from MeOH and AcOEt). $[\alpha]_D^{22}$ $-1.9°$ ($c=0.8$, 80% EtOH). IR ν_{max}^{KBr} cm^{-1}: 3370 (indole NH). MS *m/e*: 324 (M^+ $C_{19}H_{20}N_2O_3$), 280 ($M-CO_2$), 250 (280$-CH_2NH_2$), 121 (MBzl). NMR (CD_3OD): 3.70 (3H, s, OMe), 4.10 (2H, s, benzyl H), 6.65—7.80 (8H, m, aromatic H). NMR (DMSO-d_6) δ: 3.68 (3H, s, OMe), 4.07 (2H, broad s, benzyl H), 6.65—7.77 (8H, m, aromatic H), 10.8 (1H, s, indole NH). *Anal.* Calcd. for $C_{19}H_{20}N_2O_3 \cdot 1/4H_2O$: C, 69.38; H, 6.28; N, 8.52. Found: C, 69.15; H, 6.21; N, 8.03.

H–Trp(2′,5′-di-MBzl)–OH (M_3): mp 189—190° (recrystallized twice from MeOH and ether). $[\alpha]_D^{22}$ $-8.9°$ ($c=0.6$, 80% EtOH). IR ν_{max}^{KBr} cm^{-1}: 3380 (indole NH). MS *m/e*: 444 (M^+ $C_{27}H_{28}N_2O_4$), 400 ($M-CO_2$), 370 (400$-CH_2NH_2$), 121 (MBzl). NMR (CD_3OD) δ: 3.72 (6H, s, 2×OMe), 3.95 and 4.10 (2H each, broad s, 2×benzyl H), 6.65—7.58 (11H, m, aromatic H). NMR (DMSO-d_6) δ: 3.68 (6H, s, 2×OMe), 3.90 (2H, s, benzyl H), 4.05 (2H, broad s, benzyl H), 7.45 (1H, broad s, 4′-H), 10.7 (1H, s, indole NH). *Anal.* Calcd. for $C_{27}H_{28}N_2O_4 \cdot 1/2H_2O$: C, 71.50; H, 6.45; N, 6.18. Found: C, 71.53; H, 6.30; N, 5.93.

H–Trp(2′,5′,7′-tri-MBzl)–OH (M_4): mp 189—191° (recrystallized from MeOH and ether). IR ν_{max}^{KBr} cm^{-1}: 3380 (indole NH). MS *m/e*: 564 (M^+ $C_{35}H_{36}N_2O_5$), 520 ($M-CO_2$), 490 (520$-CH_2NH_2$), 121 (MBzl). NMR spectrum could not be determined, because of insufficiency of the material. From the mass spectral data, the above structure was assigned as the most plausible formula.

Isolation of the by-products derived from Boc–Trp–OH: Boc–Trp–OH (3.04 g, 10 mmol) was similarly treated with TFA (5.0 ml) in the presence of anisole (2.2 ml, 2 equiv.) containing 2% EDT and four products were isolated by repeated column chromatography on silica (2.0×35 cm) using the solvent system of $CHCl_3$–

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15) H. Yajima, H. Ogawa, and H. Sakurai, *J. Chem. Soc. Chem. Commun.*, **1977**, 909.

16) G.W. Kenner, G.A. Moore, and R. Ramage, *Tetrahedron Lett.*, **1976**, 3623.

17) M. Ueki and S. Ikeda, *Chem. Lett.*, **1976**, 827; *idem. ibid.*, **1977**, 869.

MeOH–H_2O (8: 3: 1) as stated above; B_1 24 mg (Rf_1 0.31), B_2 50 mg (Rf_1 0.40), B_3 10 mg (Rf_1 0.45) and B_4 1 mg (Rf_1 0.51).

A mixture of Three Mono-substituted Tryptophan Derivatives (B_1): mp 205—210° (recrystallized from MeOH and *n*-hexane). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3380 (indole NH). MS *m/e*: 260 (M^+ $C_{15}H_{20}N_2O_2$). NMR (CD_3-OD) δ: 1.35 (s, 2′ or 7′-Bu^t), 1.37 (s, 5′-Bu^t), 1.47 (s, 2′ or 7′-Bu^t), 7.04—7.82 (m, aromatic H).

H–Trp(1′-Bu^t)–OH (B_2): mp 188—190° (recrystallized twice from MeOH and AcOEt). $[\alpha]_D^{24}$ −30.4° (c=1.7, 80% EtOH), (lit.[10] mp 177—178°, $[\alpha]_D^{20}$ −31.2° in 80% EtOH; lit.[11] mp 179°, $[\alpha]_D$ −28.9° in 80% EtOH). IR (KBr): No indole NH band. MS *m/e*: 260 (M^+ $C_{15}H_{20}N_2O_2$), 186 ($M-C_2H_4NO_2$), 130 ($186-C_4H_8$). NMR (CD_3OD) δ: 1.72 (9H, s, Bu^t), 6.86—7.28 (2H, m, 5′,6′-H), 7.33 (1H, s, 2′-H), 7.53—7.80 (2H, m, 4′,7′-H).

H–Trp(1′,5′-di-Bu^t)–OH (B_3): mp 203—204° (recrystallized from MeOH and AcOEt). IR (KBr): No indole NH band. MS *m/e*: 316 (M^+ $C_{19}H_{28}N_2O_2$), 242 ($M-C_2H_4NO_2$), 186 ($242-C_4H_8$), 171 ($186-CH_3$). NMR (CD_3OD) δ: 1.38, 1.39 and 1.72, 1.73 (two pairs of singlets, total 9H each, $2\times Bu^t$), 7.10—7.72 (4H, m, aromatic H). These aromatic proton signals were splitted to a complicated pattern depending on equilibria of two different conformers which were revealed by the splitting of Bu^t signals, although the structures of these conformers have not been embodied.

H–Trp(2′,5′,7′-tri-Bu^t)–OH (B_4): MS *m/e*: 372 (M^+ $C_{23}H_{36}N_2O_2$), 298 ($M-C_2H_4NO_2$). Further examination were unable, because of insufficiency of the product.

Examination of Skatole as a Cation Scavenger——A mixture of skatole (0.26 g) and Z(OMe)–Gly–OH (0.24 g) was treated with TFA (1.0 ml) in an ice-bath for 60 min. The products were examined by TLC in the solvent system of $CHCl_3$ and stained by Ehrlich and $Ce(SO_4)_2$ reagents. In addition to skatole (*Rf* 0.76), at least two extra spots were detected, *Rf* 0.54 (main) and 0.39.

Amounts of the By-product derived from Boc-Trp-OH and Z(OMe)-Trp-OH——Boc–Trp–OH and Z(OMe)–Trp–OH (10 μmol each) were treated with TFA (0.2 ml) or 4 N ESA in TFE (0.2 ml) in the presence of cation scavengers (10 equiv. each) in an ice-bath for 60 min and each solution, after treatment with Amberlite CG-4B (acetate form), was examined by TLC in the solvent system of *n*-BuOH–AcOH–pyridine–H_2O (4: 1: 1: 2). Respective ninhydrin color intensities were measured by a Shimadzu dual TLC scanner at 510 mμ and the results were listed in Table I and II.

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