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Amino Acids and Peptides. XVI. Synthesis of N^G-Tosylarginyl Peptide Derivatives–Observation of Lactam Formation of Arginyl Residue[1,2]

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Z–Arg(Tos)–Pro–NHNHBoc (**1**) and Z–Arg(Tos)–Val–NHNHBoc (**2**) were prepared by the DCC, DCC–HOBt, DCC–DNp, mixed anhydride and DPPA methods. In each coupling reaction, lactam formation from the N^G-tosylarginyl residue was observed, although the extent of formation was different depending not only on the carboxyl activation method but also on the kind of amino component. Addition of HOBt suppressed the formation of acylurea but did not suppress the formation of the lactam in the synthesis of **2**. Addition of DNp suppressed the formation of the lactam slightly although it did not improve the yield of the target peptides. In the mixed anhydride method, a fairly large amount of the lactam was obtained in the synthesis of Z–Arg(Tos)–Pro–NHNHBoc, and a urethan-type derivative, N^{α}-isobutyloxycarbonyl-Pro–NHNHBoc, was also formed. However, in the case of synthesis of Z–Arg(Tos)–Val–NHNHBoc, lactam formation was suppressed compared with other activation methods and a small amount of urethan-type derivative was obtained. In both cases, the DPPA method gave a fairly good yield of the target peptide with only a trace amount of lactam formation.

Keywords——N^G-tosylarginyl peptide; chemical synthesis; activation method; lactam formation; isolation; identification

It is well known that the earliest method of protection of the guanidino function of arginine was stimulated by the availability of nitroarginine.[3] However, the nitroguanidine group has sufficient nucleophilic character to react with intramolecular electrophilic centers, resulting in formation of a lactam, a derivative of 2-piperidone.[4] In fact, the lactam derived from Z–Arg(NO_2)–OH was isolated previously.[5] Although substitution with electron-withdrawing arylsulfonyl groups renders the guanidine less sensitive to nucleophiles such as hydrazine or ammonia, lactam formation is not still impeded in N^G-tosylarginine.[6] This paper describes syntheses of N^G-tosylarginyl peptides and isolation of the lactam derived from Z–Arg(Tos)–OH by activation of the carboxyl group.

In order to prepare active fragments of kininogen and angiotensinogen, Z–Lys(Z)–Arg(Tos)–Pro–NHNHBoc (**3**) and Z–Asp(OBzl)–Arg(Tos)–Val–NHNHBoc (**4**), respectively, were required. During the synthesis of the peptides described above, several methods were used to prepare N^G-tosylarginyl peptides. Z–Arg(Tos)–OH was coupled with H–Pro–NHNHBoc or H–Val–NHNHBoc to afford Z–Arg(Tos)–Pro–NHNHBoc (**1**) or Z–Arg(Tos)–Val–NHNHBoc (**2**), respectively. However, besides the desired peptides, the piperidone derivative from Z–Arg(Tos)–OH was obtained in every case, although the extent of its formation depended not only on the carboxyl activation method but also on the N-terminal amino acid residue of the N-component. In each coupling reaction, crude products were purified by silica gel column chromatography. The DCC method in the synthesis of

TABLE I. Yield of N^G-Tosylarginyl Peptide Derivatives and Lactam

Carboxyl component	Amino component	Activation method	Yield of products (%)[a]	
			Desired peptide	Lactam
Z–Arg(Tos)–OH	H–Pro–NHNHBoc	DCC	41.2	5.0
		DCC–HOBt	74.2	trace
		DCC–DNp	42.9	2.2
		Mixed anhydride	48.6	10.0
		DPPA	83.9	trace
	H–Val–NHNHBoc	DCC	38.6	trace
		DCC–HOBt	43.3	13.0
		DCC–DNP	47.2	trace
		Mixed anhydride	50.0	trace
		DPPA	82.0	trace

a) Yield was calculated on the basis of the amount of Z–Arg(Tos)–OH used.

Z–Arg(Tos)–Pro–NHNHBoc (**1**) and the DCC–HOBt method in the synthesis of Z–Arg(Tos)–Val–NHNHBoc (**2**) gave fairly large amounts of the lactam in a crystalline form. In the latter case, addition of HOBt did not suppress the formation of the lactam but increased it slightly. The DCC–DNp[7] method, where Z–Arg(Tos)–OH was activated before combining with the N-component, gave a small amount of lactam in a crystalline form besides the desired peptide. In the mixed anhydride procedure to prepare **1**, not only the lactam from Z–Arg(Tos)–OH but also the urethan-type product, N^{α}-isobutyloxycarbonyl-Pro–NHNH-Boc,[8,9] was obtained, besides the desired product. On the other hand, in the coupling of Z–Arg(Tos)–OH with H–Val–NHNHBoc by the mixed anhydride procedure, formation of the lactam and urethan-type products was only slight. The DPPA method was also used for the preparation of **1** and **2**. It was found that the coupling of Z–Arg(Tos)–OH with H–Pro–NHNHBoc or H–Val–NHNHBoc was achieved most effectively by the DPPA method. These results are summarized in Table I.

After removal of the Z group of **1** or **2** by catalytic hydrogenation, Z–Lys(Z)–ONp or Z–Asp(OBzl)–ONp was coupled, respectively, and the crude product was purified by silica gel column chromatography to give Z–Lys(Z)–Arg(Tos)–Pro–NHNHBoc (**3**) or Z–Asp(OBzl)–Arg(Tos)–Val–NHNHBoc (**4**) in a pure form to construct the active fragment of kininogen or angiotensinogen.

In the synthesis of N^G-tosylarginyl peptides, it must be considered that lactam formation from Z–Arg(Tos)–OH is inevitable during peptide bond formation by activation of the carboxyl group of Z–Arg(Tos)–OH. The extent of its formation depends not only on the carboxyl activation method employed but also on the amino acid residue of the N-component. The lactam is fairly readily crystallizable from MeOH, and separation of the lactam derivative from the desired peptide by recrystallization is difficult.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-180 (Japan Spectroscopic Co., Ltd.). Amino acid compositions of acid hydrolysates (6 N HCl, 110 °C, 18 h) were determined with an amino acid analyzer (K-101 AS; Kyowa Seimitsu Co., Ltd.). For column chromatography, Kieselgel 60 (70—230 mesh, Merck) was used. On thin-layer chromatography (Kieselgel G, Merck), Rf^1, Rf^2 and Rf^3 values refer to the systems of $CHCl_3$ and MeOH (98 : 2), AcOEt and EtOH (4 : 1) and $CHCl_3$, MeOH and AcOH (90 : 8 : 2), respectively.

Z–Arg(Tos)–Pro–NHNHBoc (1)——1) DCC Method: Z–Arg(Tos)–OH (2.5 g) and H–Pro–NHNHBoc (pre-

pared from 2.0 g of Z-Pro-NHNHBoc[10] by catalytic hydrogenation) were dissolved in DMF (30 ml), and the solution was cooled with ice-salt. DCC (1.2 g) was added to the cold solution, and the reaction mixture was stirred at room temperature for 24 h. After removal of the urea derivative and the solvent, the residue was extracted with AcOEt. The extract was washed with 1 N HCl, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Addition of ether and petroleum ether gave a solid mass. The crude product in $CHCl_3$ (5 ml) was applied to a column of silica gel (3.5 × 10 cm), which was eluted first with $CHCl_3$ (300 ml) and then with 2% MeOH in $CHCl_3$. 1-Tosylguanyl-3-benzyloxycarbonylaminopiperidone-2 (lactam) was eluted with the latter solvent (1—150 ml), and the product was recrystallized from MeOH, mp 157—158 °C, $[\alpha]_D^{25}$ −19.7° (c=0.9, MeOH), Rf^1 0.73. *Anal.* Calcd for $C_{21}H_{24}N_4O_5S$: C, 56.8; H, 5.44; N, 12.6. Found: C, 56.7; H, 5.35; N, 12.6. Further elution with the same solvent (200—450 ml) gave the desired dipeptide, which was recrystallized from AcOEt and petroleum ether; sintering at 105 °C and melting at 120 °C, $[\alpha]_D^{25}$ −61.4° (c=0.6, MeOH), Rf^1 0.05, Rf^2 0.74. *Anal.* Calcd for $C_{31}H_{43}N_7O_8S$: C, 55.3; H, 6.43; N, 14.6. Found: C, 55.6; H, 6.73; N, 14.1.

2) DCC-HOBt Method: Z-Arg(Tos)-OH (2.5 g), H-Pro-NHNHBoc (prepared from 2.0g of Z-Pro-NHNHBoc) and HOBt (0.73 g) were dissolved in DMF (30 ml) and the solution was cooled with ice-salt. DCC (1.2 g) was added to the above cold solution. The reaction mixture was stirred at room temperature for 24 h. Isolation and purification of the products were carried out in the same way as described above. With $CHCl_3$, a trace amount of the lactam was obtained. With 1% MeOH in $CHCl_3$ (20—300 ml), *N*-[Z-Arg(Tos)]-urea was obtained, yield 0.1 g (2.7%), Rf^2 0.85, Rf^3 0.90. Further elution with the same solvent (500—1000 ml) and 2% MeOH in $CHCl_3$ (1—1000 ml) gave the desired peptide, mp 120 °C, Rf^1 0.05, Rf^2 0.75.

3) DCC-DNp Method: Z-Arg(Tos)-OH (2.4 g) and 2,4-dinitrophenol (1.0 g) were dissolved in DMF (30 ml) and the solution was cooled with ice-salt. DCC (1.2 g) was added to the above cold solution and the reaction mixture was stirred at room temperature for 3 h. After removal of the urea derivative, the filtrate was added to a cold solution of H-Pro-NHNHBoc (prepared from 2.0 g of Z-Pro-NHNHBoc) in DMF (20 ml). After 48 h, the crude products were obtained and purified in the same way as described above. Elution with 2% MeOH in $CHCl_3$ (25—175 ml) gave the lactam, which was recrystallized from MeOH, mp 156—158 °C, Rf^1 0.73. Further elution with the same solvent (200—450 ml) gave the desired peptide, sintering at 103 °C and melting at 118 °C, Rf^1 0.05, Rf^2 0.73.

4) Mixed Anhydride Method: A mixed anhydride (prepared from 2.6 g of Z-Arg(Tos)-OH, 0.6 ml of *N*-methylmorpholine and 0.72 ml of isobutyl chloroformate) in DMF (30 ml) was added to a solution of H-Pro-NHNHBoc (prepared from 2.0 g of Z-Pro-NHNHBoc) in DMF (20 ml). The reaction mixture was stirred at room temperature overnight. Isolation and purification of the products were performed in the same way as described above. Elution with 2% MeOH in $CHCl_3$ (25—200 ml) gave the lactam, which was recrystallized from MeOH, mp 157—158 °C, Rf^1 0.74. Further elution with the same solvent (200—350 ml) gave N^a-isobutyloxycarbonyl-Pro-NHNHBoc, yield 0.25 g (14.3%), mp 58—60 °C, $[\alpha]_D^{25}$ −68.8° (c=0.8, MeOH), Rf^1 0.48. *Anal.* Calcd for $C_{15}H_{27}N_3O_5$: C, 54.7; H, 8.26; N, 12.8. Found: C, 55.0; H, 8.00; N, 12.4. Further elution with the same solvent (360—660 ml) gave the desired product, mp 115—120 °C, Rf^1 0.05, Rf^2 0.73.

5) DPPA Method: Z-Arg(Tos)-OH (2.5 g) and H-Pro-NHNHBoc (prepared from 2.0g of Z-Pro-NHNHBoc) were dissolved in DMF (30 ml) and the solution was cooled with ice-salt. DPPA (1.64 g), followed by Et_3N (1.67 ml), was added and the reaction mixture was stirred at 4 °C for 24 h. Isolation and purification of the products were performed in the same way as described above. Elution with 1% MeOH in $CHCl_3$ (250—700 ml) gave trace amounts of the lactam. Elution with 2% MeOH in $CHCl_3$ (50—1500 ml) yielded the desired peptide, mp 118 °C, Rf^1 0.05, Rf^2 0.75.

Z-Arg(Tos)-Val-NHNHBoc (2)——1) DCC Method: Z-Arg(Tos)-OH (3.2 g) and H-Val-NHNHBoc (prepared from 2.5 g of Z-Val-NHNHBoc[11] were dissolved in DMF (30 ml) and the solution was cooled with ice-salt. DCC (1.7 g) was added, and the reaction mixture was stirred at room temperature for 24 h. Isolation and purification of the products were carried out in the same way as described above. Elution with 1% MeOH in $CHCl_3$ (1—250 ml) gave a trace amount of the lactam. Further elution with the same solvent (270—750 ml) gave *N*-[Z-Arg(Tos)]-urea, yield 1.58 g (34.2%), mp 105—108 °C, $[\alpha]_D^{25}$ +14.4° (x=1.1, MeOH), Rf^2 0.85, Rf^3 0.90. *Anal.* Calcd for $C_{34}H_{48}N_6O_6S$: C, 61.1; H, 7.24; N, 12.6. Found: C, 61.0; H, 7.20; N, 12.4. Elution with 2% MeOH in $CHCl_3$ (50—750 ml) yielded the desired peptide, mp 114—120 °C, $[\alpha]_D^{25}$ −30.2° (c=0.6, MeOH), Rf^1 0.05, Rf^2 0.75. *Anal.* Calcd for $C_{31}H_{45}N_7O_8S$: C, 55.1; H, 6.71; N, 14.5. Found: C, 55.5; H, 7.00; N, 14.0.

2) DCC-HOBt Method: Z-Arg(Tos)-OH (6.4 g), H-Val-NHNHBoc (prepared from 5.0 g of Z-Val-NHNHBoc) and HOBt (1.85 g) were dissolved in DMF (50 ml) and the solution was cooled with ice-salt. DCC (3.4 g) was added, and the reaction mixture was stirred at room temperature for 24 h. Crude products were purified by silica gel column chromatography in the same manner as described above. Elution with $CHCl_3$ (350 ml) gave the lactam, mp 157—158 °C, Rf^1 0.74. Elution with 2% MeOH in $CHCl_3$ (370—970 ml) gave the desired peptide, and the product was recrystallized from AcOEt and petroleum ether, Rf^1 0.05, Rf^2 0.74.

3) DCC-DNp Method: Z-Arg(Tos)-OH (3.2 g) and 2,4-dinitrophenol (1.31 g) were dissolved in DMF (20 ml) and the solution was cooled with ice-salt. DCC (1.57 g) was added to the above cold solution. The reaction mixture was stirred at room temperature for 3 h. This solution was combined with H-Val-NHNHBoc (prepared from 2.5 g of Z-Val-NHNHBoc) in DMF (10 ml). After 48 h at room temperature, the products were isolated in the same way as

described above. Elution with $CHCl_3$ (250—700 ml) gave a trace amount of the lactam, Rf^1 0.73. Elution with 1% MeOH in $CHCl_3$ (1—700 ml) yielded a small amount of acylurea, Rf^2 0.84. Elution with 2% MeOH in $CHCl_3$ (200—1000 ml) gave the desired peptide, mp 110—118 °C, Rf^1 0.05.

4) Mixed Anhydride Method: Mixed anhydride (prepared from 8.3 g of Z–Arg(Tos)–OH, 2.3 ml of isobutyl chloroformate and 1.95 ml of *N*-methylmorpholine) in DMF (50 ml) was added to a solution of H–Val–NHNHBoc (prepared from 6.5 g of Z–Val–NHNHBoc). This reaction mixture was stirred at 0 °C overnight. The crude products were purified in the same way as described above. Elution with $CHCl_3$ (400 ml) gave a trace amount of the lactam, Rf^1 0.74. Elution with 2% MeOH in $CHCl_3$ (1—200 ml) gave a trace amount of the urethan-type product, Rf^1 0.60, followed (500—1300 ml) by the desired dipeptide derivative, mp 118—123 °C, Rf^1 0.05, Rf^2 0.75.

5) DPPA Method: Z–Arg(Tos)–OH (3.2 g) and H–Val–NHNHBoc (prepared from 2.5 g of Z–Val–NHNHBoc) were dissolved in DMF (25 ml) and the solution was cooled with ice-salt. DPPA (2.09 g), followed by Et_3N (2.12 ml), was added to the above solution. The reaction mixture was stirred in a cold room for 24 h. Elution with 1% MeOH in $CHCl_3$ (1—1000 ml) gave a trace amount of the lactam. Elution with 2% MeOH in $CHCl_3$ (200—1500 ml) yielded the desired peptide, mp 113—121 °C, Rf^1 0.05.

Z–Lys(Z)–Arg(Tos)–Pro–NHNHBoc (3)——Z–Lys(Z)–ONp (1.2 g) and H–Arg(Tos)–Pro–NHNHBoc (prepared from 1.5 g of Z–Arg(Tos)–Pro–NHNHBoc by catalytic hydrogenation) were dissolved in DMF (30 ml) containing triethylamine (0.3 ml). This reaction mixture was stirred at room temperature for 24 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 1 N HCl, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. The residue was purified by silica gel column (3.5 × 10 cm) chromatography. The column was eluted with $CHCl_3$ (300 ml), 1% MeOH in $CHCl_3$ (300 ml) and then 2% MeOH in AcOEt (400 ml). From the eluate with 2% MeOH in AcOEt, the desired peptide was obtained in a yield of 1.3 g (63%) as an amorphous powder, $[\alpha]_D^{25}$ −83.6° (c=0.6, MeOH), Rf^1 0.05, Rf^2 0.70. *Anal.* Calcd for $C_{42}H_{56}N_8O_{11}S \cdot 3.5H_2O$: C, 53.4; H, 6.72; N, 11.9. Found: C, 53.2; H, 6.47; N, 12.4. Amino acid ratios in an acid hydrolysate: Lys 1.00; Arg 0.85; Pro 1.05 (average recovery 81%).

Z–Asp(OBzl)–Arg(Tos)–Val–NHNHBoc (4)——Z–Asp(OBzl)–ONp (2.1 g) and H–Arg(Tos)–Val–NHNHBoc (prepared from 3.0 g of Z–Arg(Tos)–Val–NHNHBoc) were dissolved in DMF (30 ml) containing triethylamine (0.61 ml). The reaction mixture was stirred at room temperature for 24 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 1 N HCl, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. The residue was applied to a silica gel column (3.5 × 14 cm), which was eluted with $CHCl_3$ (300 ml), and 2% MeOH in $CHCl_3$. The latter eluate (300—700 ml) contained the desired peptide, which was recrystallized from AcOEt and petroleum ether, yield 1.8 g (43.7%), mp 115—120 °C, $[\alpha]_D^{25}$ −25.8° (c=0.2, MeOH), Rf^1 0.05, Rf^2 0.60. *Anal.* Calcd for $C_{45}H_{61}N_9O_{11}S \cdot H_2O$: C, 56.7; H, 6.66; N, 13.2. Found: C, 56.7; H, 6.75; N, 13.0. Amino acid ratios in an acid hydrolysate: Asp 1.00, Arg 0.9; Val 0.9 (average recovery 75%).

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References and Notes

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