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Amino Acids and Peptides. V. Synthesis of Amino Acid Derivatives Containing a Sulfonamide Bond¹⁾

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Amino acid derivatives containing a sulfonamide bond were synthesized and their biological activities were examined. Some sulfonyllysine derivatives exhibited fibrinolytic activity. *N*⁴-Lysylsulfanilamide was also prepared and treated with trypsin. The results suggest that a drug acylated with an amino acid might have improved solubility in water and might act as a kind of pro-drug.

Keywords—sulfonylamino acid; sulfonyllysine; sulfanilylamino acid; fibrinolytic activity; pro-drug

In general, amino acids have good solubility in water, and furthermore their salts (HCl salt, Na salt, *etc.*) are more hydrophilic. Therefore acylation of a water-insoluble drug with an amino acid and salt-formation of the acylated drug may increase the solubility in water. Administration of a water-insoluble drug by aqueous injection might then be possible, and the acylated drug might be hydrolyzed by an enzyme in the body to exhibit its medicinal effect. Therefore a drug acylated with an amino acid may work as a kind of pro-drug and may exhibit prolongation of the medicinal effect. We tested this possibility by using sulfanilamide and Lys. Sulfanilamide is sparingly soluble in water and Lys is known to be a site of trypsin cleavage. *N*^α,*N*^ε-diZ-Lys²⁾ was coupled with sulfanilamide by the DCC method³⁾ followed by hydrogenation to afford *N*⁴-lysylsulfanilamide (I). Compound I was sparingly soluble in

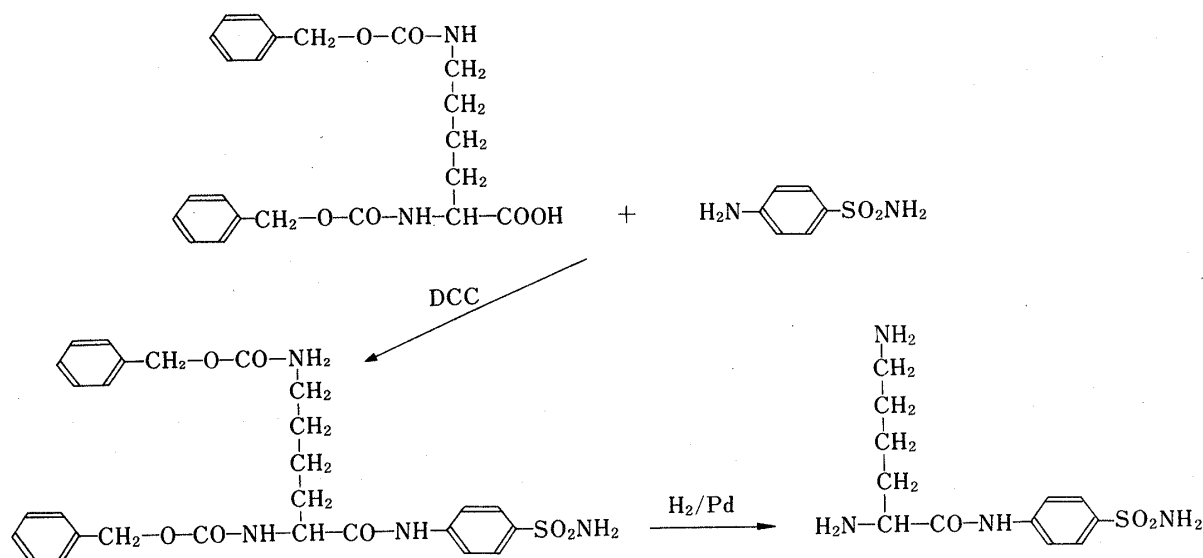


Fig. 1. Synthetic Scheme for Lysylsulfamine

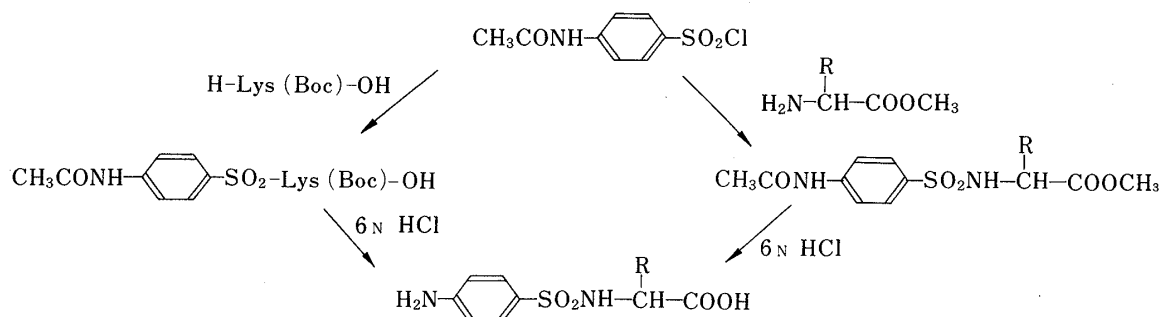


Fig. 2. Synthetic Scheme for Sulfanilylamino Acids

TABLE I. Fibrinolytic Activities of Sulfonylamino Acids

	Dose (mg/kg, i.v.)	Fibrinolytic activity (%)	
		2 min	10 min
Lys-Sulamine	10	103	116
Sulfanilyl-Lys	10	107	109
Asul-Lys(Boc)-OH	10	142	131
	5	162	131
	2.5	124	116
Asul-Lys(Z)-OH	10	150	121
	5	181	133
	2.5	166	118
Asul-Lys(Asul)-OH	1.25	124	110
	10	163	146
	5	206	153
	2.5	151	151
Dns-Lys(Boc)-OH	1.25	155	124
	0.625	158	133
	10	193	143
	5	149	120
Tos-Lys(Boc)-OH	2.5	165	121
	1.25	149	142
	10	194	144
	5	202	138
Ms-Lys(Boc)-OH	2.5	190	136
	1.25	144	124
	0.625	133	108
	10	150	102
Tos-Lys(Tos)-OH	5	103	92
	10	176	164
	5	210	126
	2.5	159	140
	1.25	169	123

$$\text{Fibrinolytic activity (\%)} = \frac{\text{euglobulin lysis time preadministration}}{\text{euglobulin lysis time postadministration}} \times 100$$

water, contrary to our expectation, but its HCl salt was readily soluble. Release of Lys was observed when I was treated with trypsin. About 60% of I was hydrolyzed by the enzyme within 24 h. The hydrolysis was slow, but this supports the view that a drug acylated with an amino acid may act as a kind of pro-drug.

Next, various amino acids were sulfanilated as shown in Fig. 2. *N*-Acetyl-sulfanilylchloride⁴⁾ was coupled with an amino acid methyl ester followed by acid hy-

drolysis with 6N HCl to afford a sulfanilylamino acid. N^α -Sulfanilyl-Lys was prepared in two different ways with a Z group⁵⁾ or Boc group⁶⁾ as the protecting group for the ϵ -amino group of Lys. N^α -Sulfanilyl-Arg was prepared by hydrogenolysis of N^α -Asul- N^G -NO₂-Arg-OMe followed by acid hydrolysis. Optical isomers of sulfanilyl-Ala were prepared; their rotations in water were -36.9° and $+36.2^\circ$. Antibacterial effects of the synthetic sulfanilylamino acids were examined but no effect was observed against various gram-positive and gram-negative organisms.

It is known that some sulfonylamino acids can affect blood coagulation. For example, some arenesulfonylarginines were reported to be thrombin inhibitors.⁷⁾ We examined the fibrinolytic activities of I, N^α -sulfanilyl-Lys and N^α -Asul- N^ϵ -Boc-Lys. Among these samples, N^α -Asul- N^ϵ -Boc-Lys exhibited fibrinolytic activity, so we prepared various sulfonyllysine derivatives to examine them for fibrinolytic activity. The sulfonyllysine derivatives were synthesized from Lys (or its derivative) and the corresponding sulfonylchloride. The synthetic sulfonyllysine derivatives and their fibrinolytic activities are summarized in Table I.

Comparing the fibrinolytic activities of these compounds, N^α -Ms- N^ϵ -Boc-Lys was less active than other N^α -arenesulfonyl- N^ϵ -Boc-lysines. An N^α -arenesulfonyl group may be more effective than an N^α -alkanesulfonyl group. Among N^α -arenesulfonyl- N^ϵ -Boc-lysines, N^α -dansyl and N^α -tosyl compounds exhibited high activity. Among N^α -Asul-lysines, N^α, N^ϵ -diAsul-Lys was the most effective, but the N^ϵ -sulfonyl group seemed not to be necessary for the activity considering the activities of N^α -sulfonyl- N^ϵ -Boc-lysines and N^α -sulfonyl- N^ϵ -Z-lysines. Some of the synthetic sulfonyllysine derivatives exhibited fibrinolytic activity but their effects were not sufficient for medical purposes. Further synthetic studies on the fibrinolytic activities of sulfonyllysine derivatives are planned.

Experimental

Melting points are uncorrected. Solvent systems for ascending thin-layer chromatography on Silica gel G (type 60, E. Merck) are indicated as follows: $Rf^1 = n\text{-BuOH-AcOH-H}_2\text{O}$ (4:1:5, upper phase), $Rf^2 = n\text{-BuOH-pyridine-AcOH-H}_2\text{O}$ (4:1:1:2), $Rf^3 = \text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (8:3:1, lower phase), $Rf^4 = \text{AcOEt-benzene}$ (1:1).

N^4 -(N^α, N^ϵ -diZ-Lys)sulfanilamide— N^α, N^ϵ -diZ-Lys (1.1 g) and sulfanilamide (0.45 g) were coupled in THF by

TABLE II. Yields and Physical Constants of Asul-amino Acid Methylesters

$\text{CH}_3\text{CONH}-\text{C}_6\text{H}_4-\text{SO}_2\text{R}$					
R (Yields %)	mp ($^\circ\text{C}$)	$[\alpha]_D^{25}$, $c = 1.0$	Analysis (%)		
			Calcd (Found)		
			C	H	N
L-Ala-OMe (69)	129—132	-33.6° , $t = 21$ MeOH	48.0 (47.7)	5.4 5.4	9.3 9.2)
D-Ala-OMe (62)	133—134	$+34.2^\circ$, $t = 24$ MeOH	48.0 (47.8)	5.4 5.3	9.3 9.3)
Pro-OMe (71)	117—123	-118.2° , $t = 26$ MeOH	51.5 (51.5)	5.6 5.7	8.6 8.4)
N^ϵ -Z-Lys-OMe (52)	145—147	$+2.4^\circ$, $t = 25$ MeOH	55.8 (56.0)	6.0 5.9	8.6 8.3)
Asp-(OMe) ₂ (67)	113—114	-1.6° , $t = 26$ MeOH	46.9 (46.6)	5.1 5.4	7.8 7.5)
N^G -NO ₂ -Arg-OMe (55)	211—213	-10.0° , $t = 26$ DMF	41.2 (41.3)	5.1 5.2	16.2 16.3)

the DCC method³⁾ in the usual manner. Recrystallized from AcOEt. Yield 1.11 g (74%), mp 177—179 °C, $[\alpha]_D^{26} -6.4^\circ$ ($c=1.0$, MeOH), Rf^3 0.64, Rf^4 0.41. Anal. Calcd for $C_{28}H_{32}N_4O_7S$: C, 59.1; H, 5.7; N, 9.9. Found: C, 59.4; H, 5.8; N, 9.7.

***N*⁴-Lysylsulfanilamide (I)**—Prepared by hydrogenation of *N*⁴-(*N*^ε,*N*^ε-diZ-Lys)sulfanilamide (1 g) over a Pd catalyst in MeOH in the usual manner. Recrystallized from MeOH. Yield 375 mg (71%), mp 171—174 °C, $[\alpha]_D^{28} +51.9^\circ$ ($c=1.0$, 1 N HCl), Rf^1 0.26, Rf^2 0.40. Anal. Calcd for $C_{12}H_{20}N_4O_3S$: C, 48.0; H, 6.7; N, 18.6. Found: C, 47.8; H, 6.9; N, 18.3.

Trypsin Treatment of I—To increase its solubility in water, I was converted into the dihydrochloride salt by lyophilization from 1 N HCl. The hydrochloride salt was treated with trypsin according to the procedure reported by Hirs *et al.*⁸⁾ Liberated Lys was analyzed periodically and the following results were obtained.

Time (h)	2	5	10	24
Liberated Lys	26%	48%	56%	60%

General Procedure for Preparation of Asul-amino Acid Methyl Esters—An amino acid methyl ester hydrochloride (0.03 mol) was dissolved in a mixture of DMF and H₂O. The solution was adjusted to pH 9 with Et₃N and combined with *N*-acetylsulfanilylchloride (0.036 mol) at 0 °C. The reaction mixture was stirred overnight at room temperature and the solvent was evaporated off. The residue was dissolved in CHCl₃ and H₂O, and the CHCl₃ layer was washed successively with 5% NaHCO₃, H₂O, 10% citric acid and H₂O. The CHCl₃ layer was dried over Na₂SO₄ and evaporated down. The residue was recrystallized from AcOEt or MeOH. Yields and physical constants of the products are shown in Table II.

***N*^ε-Asul-*N*^ε-Boc-Lys**—Prepared from *N*^ε-Boc-Lys (2.46 g), *N*-acetylsulfanilyl chloride (2.8 g) and Et₃N (3 ml) in a mixture of DMF and H₂O. Recrystallized from a mixture of AcOEt and MeOH. Yield 1.8 g (41%), mp 172—175 °C, $[\alpha]_D^{25} +19.2^\circ$ ($c=1.0$, MeOH), Rf^3 0.71, Rf^4 0.16. Anal. Calcd for $C_{19}H_{29}N_3O_7S$: C, 51.5; H, 6.6; N, 9.5. Found: C, 51.2; H, 6.6; N, 9.5.

General Procedure for Preparation of Sulfanilylamino Acids—Asul-amino acid methyl ester (1 g) was suspended in 6 N HCl (5 ml) and the mixture was kept at 100 °C for 40 min. The mixture became a clear solution, which was diluted with H₂O, washed with AcOEt and evaporated down. The residue was recrystallized from a small amount of H₂O. Asul-Asp was purified by Dowex 50 (H⁺) column chromatography using the pH gradient method with pyridine-acetate buffer (0.1 mol, pH 3.8—5.8). The nitro group on the Arg derivative was removed by hydrogenation in dioxane before hydrolysis. *N*^ε-Sulfanilyl-Lys was prepared by hydrolysis of *N*^ε-Asul-*N*^ε-Boc-Lys and *N*^ε-Asul-*N*^ε-Z-Lys-OMe. The products were identical. Yields and physical constants of the products are shown

TABLE III. Yields and Physical Constants of Sulfanilylamino Acids

$\text{HCl} \cdot \text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{SO}_2\text{R}$					
R (Yields %)	mp (°C)	$[\alpha]_D^t$, $c=1.0$	Analysis (%)		
			Calcd (Found)		
			C	H	N
L-Ala (63)	128—133	−36.9°, $t=27$ H ₂ O	+H ₂ O 36.2	5.1	9.4
			(36.1)	5.2	9.3)
D-Ala (59)	126—131	+36.2°, $t=27$ H ₂ O	+H ₂ O 36.2	5.1	9.4
			(36.1)	5.1	9.2)
Pro (68)	133—135	−93.5°, $t=25$ MeOH	43.2	4.6	9.2
			(43.5)	4.7	9.5)
Lys·HCl (66)	195—212	+6.9°, $t=27$ H ₂ O	+H ₂ O 36.7	5.9	10.7
			(36.5)	5.9	10.4)
Asp (68)	169—176	−2.9°, $t=33$ 1 N HCl	+1/2H ₂ O ^{a)} 40.4	4.4	9.4
			(40.6)	4.2	9.5)
Arg·HCl (60)	140—153	+5.7°, $t=27$ H ₂ O	+2H ₂ O 32.9	5.8	10.2
			(32.6)	6.0	10.0)

a) Not HCl salt. Purified by ion-exchange column chromatography.

TABLE IV. Yields and Physical Constants of Sulfonyllysines

Materials (Yields %)	mp (°C)	$[\alpha]_D^{25}$, $c = 1.0$	Analysis (%)		
			Calcd	Found	
			C	H	N
$\begin{array}{c} \text{Z} \\ \\ \text{Asul-Lys} \\ (54) \end{array}$	167—170	+17.3°, $t = 26$ MeOH	55.3 (55.4)	5.7 (5.7)	8.8 (8.6)
$\begin{array}{c} \text{Asul} \\ \\ \text{Asul-Lys} \\ (51) \end{array}$	168—171	+16.6°, $t = 26$ MeOH	48.9 (48.8)	5.2 (5.3)	10.4 (10.1)
$\begin{array}{c} \text{Boc} \\ \\ \text{Dns-Lys} \\ (76) \end{array}$	214	−3.0°, $t = 23$ MeOH	57.6 (57.8)	7.0 (6.9)	8.8 (8.8)
$\begin{array}{c} \text{Boc} \\ \\ \text{Tos-Lys} \\ (63) \end{array}$	128—130	−10.6°, $t = 25$ MeOH	54.0 (54.4)	7.1 (7.0)	7.0 (7.1)
$\begin{array}{c} \text{Boc} \\ \\ \text{Ms-Lys} \cdot \text{DCHA} \\ (50) \end{array}$	188—192	−5.5°, $t = 25$ MeOH	57.0 (57.0)	9.4 (9.6)	8.3 (8.7)
$\begin{array}{c} \text{Tos} \\ \\ \text{Tos-Lys} \cdot \text{DCHA} \\ (64) \end{array}$	200—205	+34.9°, $t = 25$ MeOH	60.4 (60.4)	7.8 (7.9)	6.6 (6.7)

in Table III.

Antibacterial Effects of the Sulfanilylamino Acids—No antibacterial effect against various gram-positive and gram-negative organisms was observed even at a concentration of 100 $\mu\text{g/ml}$.

General Procedure for Preparation of Sulfonyllysine Derivatives—Lys (or a Lys derivative) was sulfonylated with the appropriate sulfonylchloride (10% excess) in a mixture of H_2O , DMF and Et_3N at pH 9. After the reaction, the solvent was evaporated off and the residue was extracted with H_2O . The aqueous layer was washed with AcOEt and acidified with citric acid. The resulting precipitate was extracted with AcOEt and the AcOEt layer was washed with H_2O , dried over Na_2SO_4 and evaporated down. The residue was precipitated from AcOEt –petroleum ether. If the precipitate was syrupy, the product was crystallized as the dicyclohexylamine salt. Yields and physical constants of the products are summarized in Table IV.

Fibrinolytic Activities of Sulfonyllysine Derivatives—The activities were measured by the euglobulin clot lysis time (ELT) method reported by Von Kaulla and Schultz.⁹⁾ Samples were administered to rats by intravenous injection.

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References and Notes

- 1) Standard abbreviations for amino acids and protecting groups are used [*J. Biol. Chem.*, **247**, 977 (1972)]. Other abbreviations include: Asul, *N*-acetylsulfanilyl; Dns, dansyl; Ms, mesyl; Tos, tosyl; DCHA, dicyclohexylamine; DCC, dicyclohexylcarbodiimide; THF, tetrahydrofuran; DMF, *N,N*-dimethylformamide.
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