rabbits were isolated and mounted in baths at 35 °C in Krebs–Henseleit buffer and aerated with 95% O_2–5% CO_2. They were driven with square-wave pulses of 10-ms duration at five times the threshold voltage. The maximum effective frequency of stimulation (maximum following frequency, MFF) was determined by gradually increasing the stimulus rate from 3 Hz (the control or basal rate) to the point where skipped beats were evident and then reducing the frequency to a rate that the atria would just follow. This rate was taken as the end point, and is essentially the reciprocal of the effective refractory period of the atrium. After determination of control MFF, the MFF reduction caused by **2**, **3**, **5**, and disopyramide were determined at concentrations of 1, 3, 13, 33, and 133 μM. The dose–response curves for all atria were plotted, and the ED_{25} values (drug concentration required to reduce MFF to 75% of its control value) were estimated. Since compounds with ability to reduce MFF were seen to reduce the force of atrial contraction (FAC) at 3 Hz, the ED_{25} for this side effect was also calculated.

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Antifungal Agents. 4.[1] Chemical Modification of Antibiotics from *Polyangium cellulosum* var. *fulvum*. Ester and Amide Analogues of Ambruticin

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A series of ester and amide analogues of ambruticin (1) was prepared. The analogues were tested against *Histoplasma capsulatum*, *Microsporum fulvum*, *Candida albicans* and *Streptococcus pyogenes*. Structure–activity relationships are described.

We previously described the isolation[3] and characterization[4,5] of two potent antifungal antibiotics elaborated by *Polyangium cellulosum* var. *fulvum*. The compounds were highly active against such medically important systemic pathogens as *Histoplasma capsulatum* and *Coccidioides immitis* in vitro[3] and in vivo[6]. In view of the success of chemical-modification programs in other areas of antimicrobial chemistry and the need for nontoxic agents to combat systemic and dermatophytic fungal disease, we undertook the synthesis of chemically modified analogues.

The goals of the program were to establish the structural features necessary for activity and perhaps point the way to the construction of simpler molecules, which would retain antifungal activity and could be readily synthesized. It was also hoped to uncover molecules with more potent activity or expanded antimicrobial spectra (activity against *Candida albicans* and bacteria). Another phase of interest would be molecules retaining antifungal activity but with reduced serum binding.

In this paper, we describe the chemical modification of ambruticin (1)[4] to give esters and amides (Table I) and the resulting effects that were observed in the in vitro antifungal and antibacterial activities.

The analogues were synthesized by the routes shown in Scheme I. Acylation of **1** gave esters **9**–**12**. Treatment of ester **2** with amines gave amides **3**–**7**. The amides were converted to the corresponding diacetates, which exhibited the expected molecular ions in their mass spectra. Treatment of ester **2** with aryl isocyanates gave carbamates **16** and **17**.

The analogues were tested against Gram-positive bacteria, including *Streptococcus pyogenes*, and fungi, including *Histoplasma capsulatum*, *Microsporum fulvum*, and *Candida albicans*. Antimicrobial testing was carried out by a standard broth dilution procedure. The results are summarized in Table I.

Conversion of acid **1** to amides **3**–**7** resulted in increased activity against *S. pyogenes*. Esterification of the hydroxyl groups in acid **1** to give compounds **10**–**12** also resulted in increased activity against this organism. The remaining analogues in Table I showed little or no antibacterial activity.

The compounds (Table I) were all inactive against *C. albicans*. In general, the analogues were active against *H. capsulatum* and *M. fulvum* unless they contained bulky substituents at strategic positions (compounds **8**, **16**, and **17**). Exceptions were the diacetates (**20**, **21**, and **23**), which were inactive. A comparison of the activities of compounds **1**–**7** and molecules in which C_1 is a ketone carbonyl[7] group indicates the absence of any well-defined electronic effect at the C_1 carbonyl group on antifungal activity. The decrease in antifungal activity (especially against *M. fulvum*) in the series **1**–**4** and **8** indicates a strong steric effect at C_1 on antifungal activity. The declining activity in the series **1** and **9**–**12** and **2** and **14**–**17** illustrates the effect of increasing bulk at positions 5 and 6. Once again, antifungal activity against *M. fulvum* appears to be more sensitive to the steric effect at C_5 and C_6, as it was with respect to the steric effect at C_1.

None of the analogues showed any advantage over acid **1** with respect to antifungal activity in the presence of serum.

The polar functions at C_1, C_5, and C_6 are important for antifungal activity, and the incorporation of bulky groups at these positions reduces activity. A differentiation of the factors necessary for maximization of antifungal activity and antibacterial activity was observed in the series **1** and

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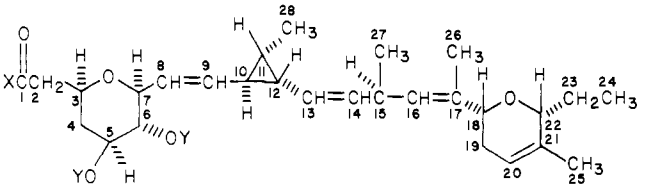
Table I. Antimicrobial Activity (in Vitro)[a,b]



no.	X	Y	yield, %	M+	MIC, μg/mL: *H. capsulatum*	*M. fulvum*	*S. pyogenes*
1[h]	OH	H		474.3009	0.195	0.098	25
2[h]	OMe	H	87	488.3240	0.39	0.39	I[c]
3	$NH(CH_2)_3CH_3$	H	52	*d*	1.56	6.25	1.56
4	$NHCH_2Ph$	H	13	*d*	6.25	>50	3.12
5	NHC_6H_{11}	H	14	*d*	3.12	25	
6	c-NC_4H_8	H	18	*d*	0.39	0.78	3.12
7	c-NC_5H_{10}	H	53	*d*	3.12	0.78	1.56
8	$N(C_6H_{11})CONHC_6H_{11}$	H	80	*e*	>50	>50	I
9	OH	COH	26	530.2930	1.56	1.56	25
10[h]	OH	COMe	60	529.2939 (M − 29)	3.12	3.12	6.25
11	OH	COEt	60	586.3193	6.25	>50	6.25
12	OH	$CO(CH_2)_2CH_3$	57	614.3643	12.5	>50	6.25
13[h]	OMe	*i* CO	69	514.2902	0.78	0.39	I
14	OMe	COH	97	544.2913	3.12	12.5	I
15[h]	OMe	COMe	68	572[f]	12.5	>50	I
16	OMe	CONHPh	81	607.3447 (M − 119)	25	>50	I
17	OMe	CONH-C_6H_4-Cl	92	612.3121 (M − 182)	50	>50	I
18	$NH(CH_2)_3CH_3$	COMe		613.3953			
19	$NHCH_2Ph$	COMe		646.3670 (M − 1)			
20	NHC_6H_{11}	COMe		639[f]	>50	>50	I
21	c-NC_4H_8	COMe		611.3861	50	>50	I
22	c-NC_5H_{10}	COMe		625.3940			
23	$N(C_6H_{11})CONHC_6H_{11}$	COMe		*g*	>50	>50	I

[a] The compounds described in this paper were either oils or gums. The homogeneity of each compound was checked by TLC in two solvent systems, ethyl acetate-cyclohexane (4:1) and ethyl acetate-2-propanol-water (85:10:5). Molecular formulas were determined by high-resolution mass spectroscopy. In cases where molecules did not give molecular ions, the composition was determined from the molecular ion of derivatives. [b] The microbiological testing procedures are described by S. M. Ringel, *Antimicrob. Agents Chemother.*, **13**, 762 (1978). [c] I, compound was inactive. [d] Molecular formulas were calculated from M+ of the corresponding diacetates. [e] Molecular formula calculated from the mass spectrum of the corresponding diacetate. [f] Only low-resolution spectra were obtained for these compounds. [g] Molecular formula calculated from fragments in the mass spectrum. [h] See ref 4. [i] Taken together.

9–12. Future papers from this laboratory will further define the structure–activity relationships in this area.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer 700 spectrometer. Mass spectra were obtained with an AEI MS-902 instrument. TLC was performed on silica gel plates (Quantum) using iodine vapor for visualization.

General Procedure for the Synthesis of Amides 3–7. A mixture of **2** (50 mg) and amine (10 mL) was heated at 110–120 °C under nitrogen for 24 h (96 h for the preparation of **4**). The excess amine was removed under reduced pressure to give an oil. The crude product was dissolved in chloroform. The chloroform solution was extracted with 1 N hydrochloric acid, washed with water, dried over $MgSO_4$, and evaporated to give a yellow oil. The product was purified by preparative TLC with the solvent system ethyl acetate–2-propanol–water (85:10:5) to give a yellow oil (homogeneous by TLC). The products showed amide bands between 1630 and 1670 cm^{-1} in the IR, and the corresponding acetates **18–22** gave the expected mass spectrum molecular ions.

General Procedure for the Synthesis of Amide Diacetates 18–22. A solution of amide (**3–7**; 5 mg) in acetic anhydride (1 mL) and pyridine (2 mL) was allowed to stand at room temperature overnight. The excess acetic anhydride was decomposed with methanol. The solvents were removed at reduced pressure to give the crude product. The product was purified by preparative TLC with the solvent system ethyl acetate–cyclohexane (4:1) to give a light brown oil (homogeneous by TLC). The products showed IR bands at 3300 (NH) (except **21** and **22**), 1740 (CO), and 1640 cm^{-1} (CO), and gave the correct molecular ions in the mass spectra.

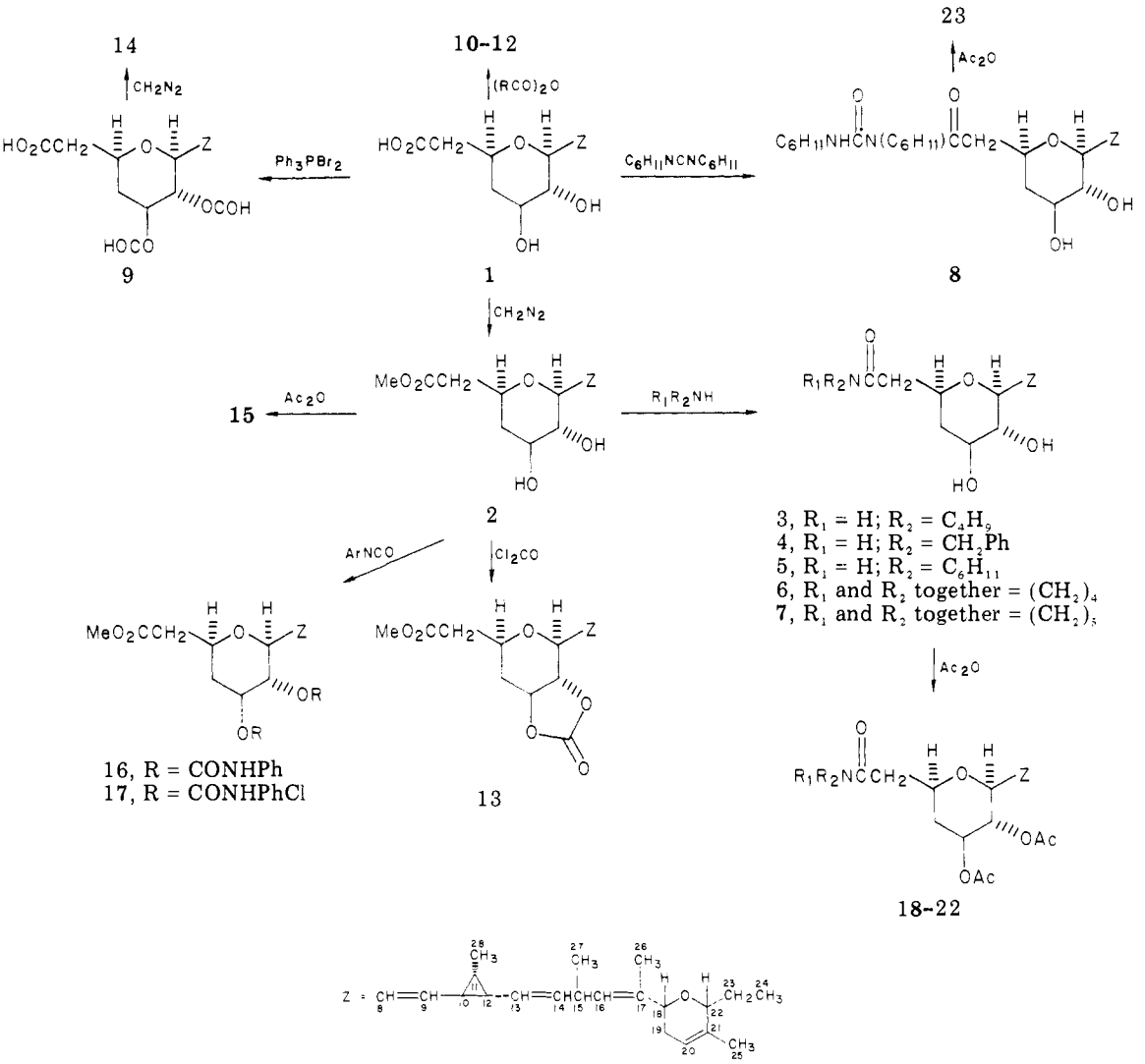
Preparation of 8. A solution of 1 (20 mg) and *N*-[(cyclohexylimino)methylene]cyclohexanamine (10 mg) in THF (10 mL) was stirred at room temperature for 5 h. The solvent was removed at reduced pressure to give a colorless oil. The oil was purified by preparative TLC with the solvent system ethyl acetate–cyclohexane (4:1) to give a colorless oil: homogeneous by TLC; yield 23 mg (80%); IR (film) 3600–3200 (OH and NH), 1700 cm^{-1} (CO).

Preparation of 23. A solution of **8** (25 mg) in acetic anhydride (1 mL) and pyridine (2 mL) was allowed to stand at room temperature overnight. Methanol was added to decompose the excess acetic anhydride. The solvents were removed at reduced pressure to give a colorless oil: homogeneous by TLC; yield 25 mg; IR (film) 3400–3250 (NH), 1740 (CO), 1700 cm^{-1} (CO). The mass spectrum of **23** showed two molecules arising from thermal fragmentation with molecular ions at 639 ($C_{38}H_{57}NO_7$) and 125 ($C_9H_{11}NO$).

5,6-Dihydroxypolyangioic Acid, Diformate (9). A solution of **1** (118 mg) in DMF (1 mL) was added to a solution of triphenylphosphine (262 mg) and bromine (160 mg) in DMF (2.5 mL) at 0 °C under nitrogen with stirring. The resulting solution was stored at 0 °C for 3 days. The reaction mixture was poured into saturated brine (10 mL) and extracted with ether (3 × 10 mL). The extracts were dried over $MgSO_4$ and evaporated to give a brown gum. The product was isolated by preparative TLC with the solvent system ethyl acetate–cyclohexane (4:1) to give a colorless oil: homogeneous by TLC; yield 35 mg, (26%); IR (film) 2700–2350 (OH), 1740 (CO), 1720 cm^{-1} (CO); MS Found: M+ 530.2930; $C_{30}H_{42}O_8$ requires 530.2878.

5,6-Dihydroxypolyangioic Acid, Dipropanoate (11). A solution of **1** (20 mg) in propionic anhydride (1 mL) and pyridine

Scheme I



(2 mL) was allowed to stand at room temperature overnight. Water (2 mL) was added and the solvents were removed at reduced pressure to give the crude product. The product was purified by preparative TLC with the solvent system ethyl acetate–cyclohexane (4:1) to give a colorless gum: homogeneous by TLC; yield 15 mg (60%); IR (film) 2800–2300 (OH), 1745 (CO), 1720 cm^{-1} (CO); MS *m/e* (relative intensity) 586 (9), 568 (2), 557 (33), 491 (11), 193 (100). Found: M^+ 586.3193; $C_{34}H_{50}O_8$ requires 586.3505.

5,6-Dihydroxypolyangioic Acid, Dibutanoate (12). A solution of 1 (20 mg) in butyric anhydride (1 mL) and pyridine (2 mL) was allowed to stand at room temperature overnight. The reaction was worked up as described for the preparation of 11 to give a colorless oil: homogeneous by TLC; yield 15 mg (57%); IR (film) 2800–2400 (OH), 1745 (CO), 1720 cm^{-1} (CO); MS *m/e* (relative intensity) 614 (10), 585 (20), 519 (10), 245 (30), 215 (90), 193 (100). Found: M^+ 614.3643; $C_{36}H_{54}O_8$ requires 614.3818.

Methyl 5,6-Dihydroxypolyangioate, Diformate (14). Excess diazomethane in ether was added to a solution of 9 (11 mg) in methanol (2 mL). The reaction mixture was allowed to stand at room temperature for 15 min. A few drops of acetic acid were added to decompose the excess diazomethane. The solvents were evaporated at reduced pressure to give a yellow oil: homogeneous by TLC; yield 11 mg; IR (film) 1740 cm^{-1} (CO); MS *m/e* (relative intensity) 544 (20), 515 (100), 449 (70), 431 (8), 419 (14), 402 (15), 357 (40). Found: M^+ 544.2913; $C_{31}H_{44}O_8$ requires 544.3039.

Preparation of 16. A solution of 2 (20 mg) and phenyl isocyanate (30 mg) in toluene (5 mL) was refluxed under nitrogen for 2 h. The solvent was removed under reduced pressure. The product was purified by preparative TLC with the solvent system ethyl acetate–cyclohexane (1:2) to give a colorless gum: homogeneous by TLC; yield 24 mg (81%); IR (film) 3400–3200 (NH), 1740 (CO), 1720 cm^{-1} (CO); MS *m/e* (relative intensity) 726 (1), 708 (1), 697 (1), 607 (11), 589 (5), 578 (13), 512 (7), 488 (5), 470 (5), 459 (10), 414 (11), 393 (8), 277 (17), 275 (16), 259 (16), 195 (26), 193 (100). Found: $M^+ - 119$, 607.3447; $C_{36}H_{49}NO_7$ requires 607.3509.

Preparation of 17. Prepared by the method described above from 2 (20 mg) and 4-chlorophenyl isocyanate (35 mg). The product was purified by preparative TLC with the solvent system ethyl acetate–cyclohexane (1:2) to give a colorless gum: homogeneous by TLC; yield 30 mg (92%); IR (film) 3400–3200 (NH), 1740 (CO), 1720 cm^{-1} (CO); MS *m/e* (relative intensity) 699 (6), 641 (24), 623 (13), 612 (100), 547 (20), 546 (20), 545 (40), 488 (30), 470 (21), 459 (100), 448 (23), 430 (11), 428 (11), 393 (40), 193 (off scale). Found: M^+ – 182, 612.3121; $C_{34}H_{43}ClNO_7$ requires 612.3039.

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Synthesis of Angiotensin II Antagonists Containing Sarcosine in Position 7

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Analogues of the type [1-sarcosine,7-sarcosine,8-X]angiotensin II, where X = isoleucine, leucine, alanine, methionine, *O*-methylthreonine, or DL-alloisoleucine, were synthesized by the solid-phase method and purified by partition chromatography, cation-exchange chromatography, and high-pressure liquid chromatography. In the isolated rat uterus, these analogues had activities of <0.1, <0.1, <0.1, <0.1, <0.1, and 0.7%, respectively, of the myotropic activity of angiotensin II and inhibited the contractile response to angiotensin II with pA_2 values of 8.1, 7.2, 6.7, 7.7, 7.4, and 8.4, respectively. In the vagotomized ganglion blocked rat, the analogues had 0.7, 0.21, 0.06, 0.72, 0.13, and 12.5%, respectively, of the pressor activity of angiotensin II.

Studies of structure–activity relationships of angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) analogues have shown that replacement of the C-terminal phenylalanine residue with a nonaromatic lipophilic residue (e.g., alanine,[1] isoleucine[2]) invokes antagonist properties to the molecule. Similarly, the substitution of a residue with a lipophilic side chain terminating in a hydrophilic functional group (e.g., methionine,[3] threonine,[4] *O*-methylthreonine[3]) or an *N*-methyl-substituted amino acid (e.g., *N*-methylisoleucine,[3] *N*-methylphenylalanine[5]) at the C terminus produces inhibitors of the myotropic and pressor responses to angiotensin II. We thought it of interest to study the penultimate amino acid at the C terminus of the molecule as a possible functional site which could be modified to produce useful analogues of angiotensin II. Earlier work has shown that replacement of the proline residue in position 7 of angiotensin II with glycine[6] or alanine[6] produces analogues with very weak activities, whereas substitution of *N*-methylalanine in position 7 results in retention of reasonable activity.[7] We have substituted sarcosine (*N*-methylglycine) in place of the proline residue normally occupying position 7 of angiotensin II. Sarcosine, like proline, can take up either the cis or the trans conformation at the peptide bond and can also produce a bend in the peptide backbone.

Results and Discussion

The results in Table I indicate that replacement of the proline residue in angiotensin II with sarcosine does not severely alter the properties of the molecule with respect to receptor interactions. [Sar^1,Sar^7]angiotensin II is a potent agonist having about one-fifth of the myotropic and pressor activities of angiotensin II and, in keeping with this, [Sar^1,Sar^7,Ile^8]antiotensin II is a potent antagonist in the isolated rat uterus. Similarly, all of the analogues studied of the type [Sar^1,Sar^7,X^8]angiotensin II, where X is a nonaromatic hydrophobic amino acid, demonstrate antagonist properties, although none of these analogues is a more potent antagonist than [Sar^1,Ile^8]angiotensin II in the rat uterus assay.

With respect to intrinsic activity, as determined on the rat uterus in vitro and the in vivo rat pressor assay, these analogues appear to follow the same general trend as many other angiotensin II antagonists; i.e., they exhibit a very low myotropic activity and a low, but significant, pressor activity. There is also a general trend with the analogues reported here for the pressor activity to parallel the in vitro antagonist potency, suggesting a dependence on binding affinity for the receptors in both situations. The only exception is [Sar^1,Sar^7,DL-aIle^8]angiotensin II, which has an unusually high agonist activity both in vivo and in vitro.

It has been predicted on the basis of conformational calculations at the receptor that the C-terminal portion of the angiotensin II molecule plays a minor role, if any, in actual binding to the receptors.[9] The results presented here could be in agreement with this possibility, since the replacement of proline by sarcosine has minimal effects on the properties of angiotensin II analogues. It is concluded that a bend produced by the presence of a secondary amino acid at position 7 of angiotensin II analogues is of critical importance in maintaining the molecule in a conformation which is recognized by angiotensin II receptors. Moreover, the decreased agonist and antagonist activities of analogues which contain the less constrained sarcosine residue in place of proline may result from a decrease in the angle of a turn[16] in the C-terminal part of the molecule, resulting in slightly less effective binding to angiotensin II receptors.

Experimental Section

tert-Butyloxycarbonyl-blocked amino acids were purchased from Bachem Inc. and examined for purity by TLC before use. A sample of Boc-aIle, specified by the manufacturer to contain only the L isomer, was found to contain equal amounts of the L and D isomers (determined by amino acid analysis after deprotection and treatment with either L- or D-amino acid acid oxidase). [Sar^1,Ile^8]angiotensin II was a product of Peninsula Labs. 1-Hydroxybenzotriazole (Aldrich) was dehydrated and purified by refluxing with benzene in a Dean and Stark reflux apparatus, followed by drying in vacuo over P_2O_5.

TLC of the analogues was carried out on silica gel on glass plates (Brinkmann Instruments, 60F-254) in the following solvent systems: *n*-butanol–pyridine–acetic acid–water (BPAW; 15:10:3:6, v/v) and chloroform–methanol–acetic acid–water (CMAW; 15:10:3:6, v/v). Thin-layer electrophoresis was conducted on cellulose on plastic sheets (Brinkmann, Polygram Cell 400 UV) in pyridine–acetate buffer, pH 6.5, at 500 V for 1 **h**. Detection of peptides on chromatograms was sequentially by UV fluoresence quenching, ninhydrin spray reagent, and chlorination followed by starch–KI spray reagent.

Boc-amino acid-resins were hydrolyzed in sealed tubes in 12 N HCl–propionic acid (1:1, v/v) at 165 °C for 15 min. Peptides

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