

Synthesis and Structure-Activity Relationships of Bestatin Analogues, Inhibitors of Aminopeptidase B

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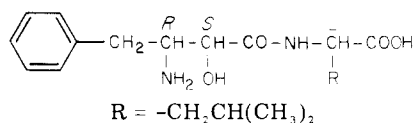
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Stereoisomers and analogues of bestatin, [(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine, were synthesized and tested for aminopeptidase B and leucine aminopeptidase inhibiting activity. Among the eight stereoisomers, the 2*S* stereoisomers exhibited strong activity. In a series of compounds in which the L-leucine residue of bestatin was substituted with other amino acids, only the one containing isoleucine showed more activity than bestatin. Norleucine, norvaline, methionine, valine, serine, glutamine, phenylalanine, glutamic acid, proline, and lysine analogues gave, in that order, decreasing activity. Alkyl and phenyl substitution for the benzyl group of bestatin decreased the activity markedly. *p*-Methyl-, *p*-chloro-, and *p*-nitrobestatins showed greater activity than bestatin.

Bestatin, which has been isolated from culture filtrates of *Streptomyces olivoreticuli*, inhibits aminopeptidase B and leucine aminopeptidase but not aminopeptidase A, carboxypeptidases, or endopeptidases.¹ Bestatin is a competitive inhibitor with K_i for aminopeptidase B of 6.0×10^{-8} and K_i for leucine aminopeptidase of 2.0×10^{-8} .



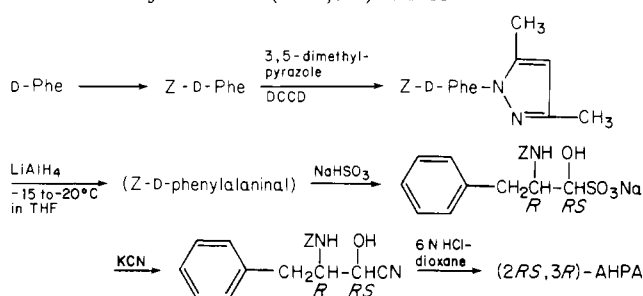
Bestatin is [(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine.² (2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutanoic acid [abbreviated as (2*S*,3*R*)-AHPA] is a new amino acid. We have synthesized stereoisomers of bestatin and related compounds and studied structure-activity relationships.

Synthesis. The method employed for the synthesis of a mixture of (2*S*,3*R*)-AHPA and (2*R*,3*R*)-AHPA is shown in Scheme I. *N*-Benzyloxycarbonyl-D-phenylalanine was coupled to 3,5-dimethylpyrazole with dicyclohexylcarbodiimide to give a crystalline product in 95% yield. It was reduced with lithium aluminum hydride in tetrahydrofuran at -15 to -20 °C. The product, *N*-benzyloxycarbonyl-D-phenylalinal, was treated with cold aqueous sodium hydrogen sulfite to yield a solid adduct which was transformed to the cyanohydrin by treatment with potassium cyanide. The cyanohydrin was hydrolyzed with 6 *N* hydrochloric acid to give a mixture of (2*R*,3*R*)- and (2*S*,3*R*)-AHPA in 55% overall yield from D-phenylalanine. In the same way, a mixture of (2*R*,3*S*)- and (2*S*,3*S*)-AHPA was prepared from L-phenylalanine. Starting from D-alanine, D-leucine, D-phenylglycine, *o*-chloro-DL-phenylalanine, *p*-chloro-DL-phenylalanine, and *p*-methyl-DL-phenylalanine, other AHPA analogues were prepared in the same way.

Resolution of the diastereoisomeric mixture of AHPA was achieved by fractional crystallization of the *N*-benzyloxycarbonyl derivatives. The erythro isomers (2*R*,3*R* and 2*S*,3*S*) were crystallized from ethyl acetate-petroleum ether, and from the mother liquors the three isomers were precipitated with brucine (2*S*,3*R* isomer) or dehydroabietylamine (2*R*,3*S* isomer). The purity of these four *N*-benzyloxycarbonyl-AHPA compounds was confirmed by oxidation with potassium permanganate to optically pure *N*-benzyloxycarbonylphenylalanine.

N-Benzyloxycarbonyl-AHPA and its analogues were coupled to amino acid benzyl esters with dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole.

Scheme I. Synthesis of (2*RS*,3*R*)-AHPA



The protecting group was removed by hydrogenation with palladium black to yield bestatin analogues. For the preparation of AHPA-methionine, *N*-*t*-Boc-AHPA was coupled with methionine methyl ester followed by alkali and trifluoroacetic acid treatments to remove the protecting groups. *p*-Nitrobestatin was prepared by nitration of bestatin and *p*-aminobestatin was prepared from *p*-nitrobestatin by catalytic hydrogenation with palladium black in methanol. Catalytic hydrogenation of bestatin with platinum oxide in acetic acid and ethanol (1:1) gave the hexahydro derivative.

Structure-Activity Relationships. Bestatin is a strong inhibitor of aminopeptidase B with a 50% inhibition concentration (IC_{50} value) of 0.05 $\mu\text{g/mL}$.¹ There are three reactive functional groups, NH_2 , OH , and COOH , in the bestatin molecule. The *N*-benzyloxycarbonyl derivative did not inhibit at 250 $\mu\text{g/mL}$ and the amide of bestatin had only very weak activity ($IC_{50} = 35 \mu\text{g/mL}$). These results indicate that the free amino group is essential for activity and the carboxyl group also is important. A role for the hydroxyl group is suggested by the activity of different stereoisomers as will be discussed later. (2*S*,3*R*)-AHPA itself showed only very weak activity ($IC_{50} = 160 \mu\text{g/mL}$) and its amide and *N*-benzyloxycarbonyl derivative did not inhibit at 250 $\mu\text{g/mL}$.

Bryce and Rabin³ proposed the reaction mechanism for leucine aminopeptidase shown in Scheme II. Bestatin shows strong inhibition of leucine aminopeptidase as well as aminopeptidase B. Binding of bestatin to the active site of these enzymes (Scheme II) is suggested by the competitive inhibition. The N-terminal amino group of the substrate corresponds to the amino group at C_3 of AHPA of bestatin but the configuration of the N-terminal amino acid of the natural substrate is *S* while the configuration at C_3 is *R*. Therefore, the eight stereoisomers of bestatin were prepared and tested for inhibition of aminopeptidase B and leucine aminopeptidase. The results shown in Table

Table I. Bestatin and Its Stereoisomers

Compd	Stereochemistry			Yield, %	R_f^a	$[\alpha]^{20-25}_{D^{578}}$ in AcOH	Formula	Analyses	Act., ^b IC ₅₀ , μg/ml	
	2	3	α						AP-B	LAP
1a	S	R	S (=L)	87.9	0.48	-23.5	C ₁₆ H ₂₄ O ₄ N ₂	C, H, N	0.05	0.01
1b	S	R	R (=D)	57.6	0.49	+0.9	C ₁₆ H ₂₄ O ₄ N ₂	C, H, N	0.56	3.4
1c	S	S	S	93.4	0.37	-61.3	C ₁₆ H ₂₄ O ₄ N ₂	C, H, N	1.25	0.07
1d	S	S	R	80.5	0.52	-31.4	C ₁₆ H ₂₄ O ₄ N ₂	C, H, N	0.05	2.7
1e	R	R	S	63.8	0.52	+30.3	C ₁₆ H ₂₄ O ₄ N ₂	C, H, N	100	7.5
1f	R	R	R	64.3	0.37	+60.2	C ₁₆ H ₂₄ O ₄ N ₂	H, N; C ^c	>250	>250
1g	R	S	S	73.5	0.49	-1.4	C ₁₆ H ₂₄ O ₄ N ₂	C, H, N	>250	>250
1h	R	S	R	58.9	0.48	+22.7	C ₁₆ H ₂₄ O ₄ N ₂	C, H, N	135	>250

^a Developing solvent, BuOH-AcOH-H₂O (4:1:1), detected by ninhydrin. ^b See ref 1 for assay methods for aminopeptidase B (AP-B) and leucine aminopeptidase (LAP). ^c C: calcd, 62.31; found, 61.77. ^d R = -CH₂CH(CH₃)₂.

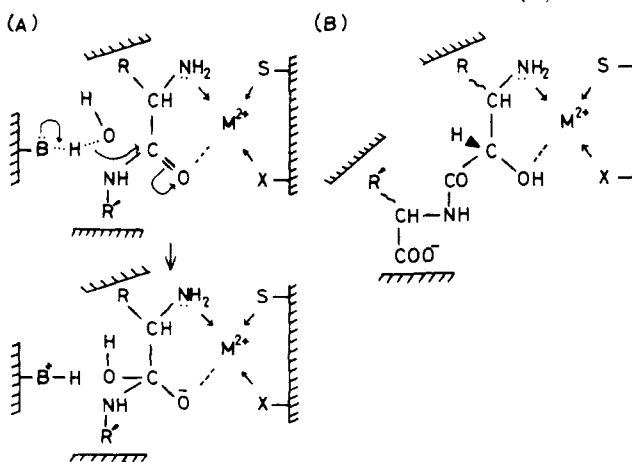
Table II. Substitution for the L-Leucine Moiety of Bestatin

Compd	R	Yield, %	R_f^a	$[\alpha]^{20-25}_{D^{578}}$ in AcOH	Formula	Analyses	Act. ^b (AP-B), IC ₅₀ , μg/ml
2	Gly	46.5 ^c	0.26	-7.4	C ₁₂ H ₁₆ O ₄ N ₂ ·CH ₃ OH	H, N; C ^d	21.5
3	β-Ala	67.7	0.34	-2.3	C ₁₃ H ₁₈ O ₄ N ₂	C, H, N	37.5
4	Gaba ^e	10.5 ^c	0.35	-14.5	C ₁₄ H ₂₀ O ₄ N ₂ ·0.5H ₂ O	C, H, N	110
5	ε-Aca ^f	78.0	0.48	-16.8	C ₁₆ H ₂₄ O ₄ N ₂	C, H, N	110
6	L-Pro	74.1	0.25	-54.1	C ₁₅ H ₂₀ O ₄ N ₂ ·H ₂ O	C, H, N	>250
7	L-Lys	88.3	0.06	-2.0	C ₁₆ H ₂₅ O ₄ N ₃ ·1.5CH ₃ OH·H ₂ O	C, H, N	>250
8	L-Glu	93.3	0.27	-19.4	C ₁₅ H ₂₀ O ₆ N ₂	C, H, N	25
9	L-Gln	51.7	0.17	-9.9	C ₁₅ H ₂₁ O ₅ N ₃ ·H ₂ O	C, H, N	1.2
10	L-Ser	66.7	0.20	-9.0	C ₁₃ H ₁₈ O ₅ N ₂ ·H ₂ O	H, N; C ^g	0.7
11	L-Met	55.0	0.37	-20.4	C ₁₅ H ₂₂ O ₄ N ₂ S·0.5H ₂ O	C, N; H ^h	0.22
12	L-Ala	79.5	0.21	-30.3	C ₁₃ H ₁₈ O ₄ N ₂	C, H, N	0.27
13	L-Val	90.5	0.42	-1.4	C ₁₅ H ₂₂ O ₄ N ₂	C, H, N	0.55
14	L-Nva	77.6	0.41	-14.5	C ₁₅ H ₂₂ O ₄ N ₂ ·0.5H ₂ O	C, H, N	0.17
15	L-Ile	73.7	0.48	+6.4	C ₁₆ H ₂₄ O ₄ N ₂	C, H, N	0.04
16	L-Nle	79.0	0.47	-8.9	C ₁₆ H ₂₄ O ₄ N ₂ ·H ₂ O	C, H, N	0.13
17	L-Phe	50.1	0.44	+0.4	C ₁₉ H ₂₂ O ₄ N ₂ ·CH ₃ OH	C, H, N	4.2
18	DL-Aoc ⁱ	49.3	0.58	-24.9	C ₁₈ H ₂₈ O ₄ N ₂	C, H, N	3.1
19	Isoamylamine hydrochloride	67.0	0.65	-18.9	C ₁₅ H ₂₅ O ₂ N ₂ Cl	C, H, N	170
20	L-Leu-Gly	51.5	0.34	-27.8	C ₁₈ H ₂₇ O ₅ N ₃	C, H, N	34

^a See footnote a, Table I. ^b See footnote b, Table I. ^c Yield from Z-AHPA. ^d C: calcd, 54.92; found, 54.33. ^e γ-Aminobutyric acid. ^f ε-Aminocaproic acid. ^g C: calcd, 51.99; found, 51.39. ^h H: calcd, 6.91; found, 6. ⁱ DL-α-Amino-octanoic acid.

I indicate that the configuration at C₂, but not that at C₃, is the most important factor for the manifestation of activity and that the stereochemical requirements for the other asymmetric carbon atoms with regard to activity are not very strict. For example, (2S,3S)-AHPA-D-Leu (1d) showed almost the same activity as bestatin against aminopeptidase B. The activity of the stereoisomers against aminopeptidase B did not correlate exactly with that against leucine aminopeptidase. This may be due to a difference of the environment around the active site in these enzymes. The fact that the S configuration at C₂ of AHPA is a requirement for enzyme inhibition suggests that in the enzymic reaction the hydration of the carbonyl group is stereospecific.

Bestatin-related compounds in which the L-leucine moiety is substituted by other amino acids, a peptide, or an amine are shown in Table II. Among those containing α-, β-, γ-, and ε-amino acids, the compound in which the amino group was α to the carboxyl group (2) showed the strongest activity. It suggests that the distance between the amino and carboxyl groups is important for enzyme inhibition. Among the compounds containing the usual

Scheme II. Reaction Mechanism of Leucine Aminopeptidase Proposed by Bryce and Rabin³ (A) and Probable Inhibition Mechanism of Bestatin (B)

α-L-amino acids, those containing proline (6) and lysine (7) showed the weakest activity. The compound containing

Table III. Substitution for the Benzyl Group of Bestatin

Compd	R	Stereo-chemistry		Yield, %	R_f^a	$[\alpha]^{20-25}_{578}$ in AcOH	Formula	Analyses	Act. ^b (AP-B), IC ₅₀ , μg
		2	3						
21a	H	<i>S</i>		78.0	0.13	-57.9	C ₉ H ₁₈ O ₄ N ₂	H, N; C ^c	140
21b	H	<i>R</i>		92.0	0.13	+8.2	C ₉ H ₁₈ O ₄ N ₂	C, H, N	> 250
22	Me	<i>RS</i>	<i>R</i>	71.6 ^d	0.23, 0.27 ^e	-28.7	C ₁₀ H ₂₀ O ₄ N ₂	C, H, N	16
23	<i>i</i> -Bu	<i>RS</i>	<i>R</i>	28.4 ^d	0.43, 0.49 ^e	-26.6	C ₁₃ H ₂₆ O ₄ N ₂	C, H, N	12
24	<i>c</i> -C ₆ H ₁₀ -Me	<i>S</i>	<i>R</i>	95.3 ^f	0.47	-30.5	C ₁₆ H ₃₀ O ₄ N ₂	C, H, N	1.1
25	Ph	<i>RS</i>	<i>R</i>	81.7	0.38, 0.41 ^e	-2.4	C ₁₅ H ₂₂ O ₄ N ₂	C, H, N	56
26	<i>p</i> -NO ₂ -Bzl	<i>S</i>	<i>R</i>	67.0 ^f	0.58	+5.2	C ₁₆ H ₁₃ O ₆ N ₃	C, H, N	0.01
27	<i>p</i> -NH ₂ -Bzl	<i>S</i>	<i>R</i>	90.5 ^g	0.25	-23.2	C ₁₆ H ₁₅ O ₄ N ₃	H, N; C ^h	0.10
28	<i>o</i> -Cl-Bzl	<i>RS</i>	<i>RS</i>	61.9	0.46	-20.8	C ₁₆ H ₁₃ O ₄ N ₃ Cl	C, H, N	0.48
29	<i>p</i> -Cl-Bzl	<i>RS</i>	<i>RS</i>	71.9	0.42, 0.52 ^e	-11.3	C ₁₆ H ₁₃ O ₄ N ₃ Cl	C, N; H ⁱ	0.07
30	<i>p</i> -CH ₃ -Bzl	<i>RS</i>	<i>RS</i>	51.5 ^d	0.53, 0.58 ^e	-7.2	C ₁₇ H ₂₆ O ₄ N ₂	C, H, N	0.01

^a See footnote a, Table I. ^b See footnote b, Table I. ^c C: calcd, 49.53; found, 50.52. ^d Yield from Z-amino acid. ^e Erythro and threo isomers could be separated. ^f Yield from 1a. ^g Yield from 26. ^h C: calcd, 59.42; found, 59.01. ⁱ H: calcd, 6.76; found, 7.29.

Table IV

Compd	R	Stereo-chemistry	Yield, %	Mp, °C	$[\alpha]^{20-25}_{578}$ in AcOH	Formula	Analyses
31a	Bzl	<i>R</i>	93.5	139	-73.0	C ₂₂ H ₂₃ O ₃ N ₃	C, H, N
31b	Bzl	<i>S</i>	92.8	142	+73.9	C ₂₂ H ₂₃ O ₃ N ₃	C, H, N
32	Me	<i>R</i>	82.4	121-122	-11.1	C ₁₆ H ₁₉ O ₃ N ₃	C, H; N ^a
33	<i>i</i> -Bu	<i>R</i>	60.4	60-61	-12.9	C ₁₉ H ₂₅ O ₃ N ₃	C, H, N
34	Ph ^b	<i>R</i>					
35	<i>o</i> -Cl-Bzl	<i>RS</i>	90.0			C ₂₂ H ₂₁ O ₃ N ₃ Cl	C, H, N
36	<i>p</i> -Cl-Bzl	<i>RS</i>	73.1			C ₂₂ H ₂₁ O ₃ N ₃ Cl	C, H, N
37	<i>p</i> -CH ₃ -Bzl	<i>RS</i>	61.7			C ₂₃ H ₂₅ O ₃ N ₃	H, N; C ^c

^a N: calcd, 13.95; found, 13.51. ^b Oily. ^c C: calcd, 70.57; found, 70.13.

isoleucine (15) was most active with equal or somewhat stronger activity than bestatin. The compounds containing amino acids with bulky side chains such as phenylalanine (17) and 2-aminooctanoic acid (18) showed weak activity. Decarboxylated bestatin which contained isoamylamine instead of L-leucine (19) and a compound containing leucylglycine (20) also showed weak activity, suggesting the involvement of the free carboxyl group of bestatin in enzyme inhibition.

The activity of bestatin-related compounds in which the benzyl group of bestatin is substituted by hydrogen, alkyl, phenyl, and substituted benzyl groups is shown in Table III. (*S*)-Isoseryl-L-leucine (21a) showed weak activity and the *R* isomer 21b was inactive. This also indicates the importance of the *S* configuration at C₂ of the AHPA for activity. The alkyl- and phenyl-substituted derivatives showed only weak activity. Among them, the cyclohexylmethyl derivative 24, that is, hexahydrobestatin, showed the strongest activity. The activity of *p*-nitrobestatin (26) was five times stronger than bestatin, but the activity of the *p*-amino derivative 27 was less than that of bestatin. To test the effect of aromatic substitution, the *o*- and *p*-chlorobestatins (diastereoisomeric mixtures) were prepared. The activity of the ortho isomer 28 was about one-tenth that of bestatin, while the para isomer 29 was slightly less active than bestatin. The content of the 2*S*,3*R* isomer in 28 and 29 was about 25%. Therefore, it is probable that *p*-chloro substitution of bestatin increased

the activity while *o*-chloro substitution decreased it. The activity of the diastereoisomeric mixture of *p*-methylbestatin (30) was 0.01 μg/mL (IC₅₀). Thus (2*S*,3*R*)-*p*-methylbestatin is probably the most active of the compounds so far prepared.

Experimental Section

Melting points were determined by a Shibata melting point apparatus and are uncorrected. Optical rotations were measured by a Perkin-Elmer 141 automatic polarimeter at 578 nm. Microanalyses were done on a Perkin-Elmer 240 microanalyzer and analytical values without an asterisk were within ±0.4% of the calculated value. TLC's were done on Merck's precoated silica gel 60 F₂₅₄ plates. Evaporations were accomplished by a rotary evaporator using aspirator vacuum. All D-amino acids were purchased from Nippon Rikagaku Yakuhin Co., Japan. Aminopeptidase B (E.C. 3.4.11.6), purified according to the method of Hopsu et al.,⁴ and L-arginine-β-naphthylamide, purchased from Protein Research Foundation, Japan, were used for assay of aminopeptidase B activity. Leucine aminopeptidase (E.C. 3.4.11.1), purchased from Miles Laboratories, Inc., and L-leucine-β-naphthylamide, purchased from Tokyo Chemical Industry Co., Ltd., Japan, were used for assay of leucine aminopeptidase activity. For details of the assay methods see ref 1.

General Synthetic Procedure of Z-Amino Acid 3,5-Dimethylpyrazolide. Z-Amino acid (10 mmol) and 3,5-dimethylpyrazole (DMP) (12 mmol) were treated with DCCD (10 mmol) in CHCl₃ (200 mL) at -10 °C for 1 h and then left at room temperature overnight. After removal of dicyclohexylurea, the solvent was evaporated. The solid residue was dissolved in EtOAc

Table V

Compd	R	Stereochemistry		Yield, %	R_f^a	Formula	Analyses
		2	3				
		$\begin{array}{c} \text{3} \quad \text{2} \\ \text{RCH-CHCOOH} \\ \quad \\ \text{H}_2\text{N} \quad \text{OH} \end{array}$					
38	Me	RS	R	72.8	0	$\text{C}_4\text{H}_9\text{O}_3\text{N}$	C, H, N
39	<i>i</i> -Bu	RS	R	64.2	0.18	$\text{C}_7\text{H}_{15}\text{O}_3\text{N}$	C, H, N
40	Ph	RS	R	8.8	0.13, 0.17 ^b	$\text{C}_9\text{H}_{13}\text{O}_3\text{N}$	H, N; C ^c
41a	Bzl	RS	R	55.2	0.23, 0.26 ^b	$\text{C}_{10}\text{H}_{13}\text{O}_3\text{N}$	H, N; C ^d
41b	Bzl	RS	S	31.7	0.23, 0.26 ^b	$\text{C}_{10}\text{H}_{13}\text{O}_3\text{N}$	C, H, N
42	<i>o</i> -Cl-Bzl	RS	RS	58.3	0.25, 0.29 ^b	$\text{C}_{10}\text{H}_{12}\text{O}_3\text{NCl}$	C, H, N
43	<i>p</i> -Cl-Bzl	RS	RS	37.0	0.25	$\text{C}_{10}\text{H}_{12}\text{O}_3\text{NCl}$	C, H, N
44	<i>p</i> -CH ₃ -Bzl	RS	RS	5.3	0.20	$\text{C}_{11}\text{H}_{15}\text{O}_3\text{N}$	C, H, N

^a See footnote a, Table I. ^b Erythro and threo isomers could be separated. ^c C: calcd, 59.66; found, 59.18. ^d C: calcd, 61.52; found, 61.06.

Table VI. Z-AHPA

Compd	Stereochemistry		Yield, %	Mp, °C	$[\alpha]^{20-25}_{578}$ in AcOH	Formula	Analyses
	2	3					
45a	S	R	67.0	154-155	+83.5	$\text{C}_{18}\text{H}_{19}\text{O}_5\text{N}$	C, H, N
45b	R	R	47.6	175-176	+5.7	$\text{C}_{18}\text{H}_{19}\text{O}_5\text{N}$	C, H, N
45c	S	S	41.3	175-176	-6.3	$\text{C}_{18}\text{H}_{19}\text{O}_5\text{N}$	H, N; C ^a
45d	R	S	36.1	154-155	-84.2	$\text{C}_{18}\text{H}_{19}\text{O}_5\text{N}$	C, H, N

^a C: calcd, 65.64; found, 65.13.

Table VII. AHPA

Compd	Stereochemistry		Yield	$[\alpha]^{20-25}_{578}$ in 1 N HCl	Formula	Analyses
	2	3				
46a	S	R	Quantitative	+29.5	$\text{C}_{10}\text{H}_{13}\text{O}_3\text{N} \cdot 1/3 \text{H}_2\text{O}$	C, H, N
46b	R	R	Quantitative	+5.2	$\text{C}_{10}\text{H}_{13}\text{O}_3\text{N}$	H, N; C ^a
46c	S	S	Quantitative	-5.6	$\text{C}_{10}\text{H}_{13}\text{O}_3\text{N}$	H, N; C ^b
46d	R	S	Quantitative	-31.0	$\text{C}_{10}\text{H}_{13}\text{O}_3\text{N} \cdot 1/3 \text{H}_2\text{O}$	C, H, N

^a C: calcd, 61.52; found, 62.01. ^b C: calcd, 61.52; found, 60.87.

and washed with 1 N HCl and H₂O. The solvent phase was dried with MgSO₄ and evaporated to give a solid residue which was crystallized from EtOAc-petroleum ether. The Z-amino acid-DMP products thus obtained are summarized in Table IV.

General Synthetic Procedure for AHPA and Its Analogues. To a suspension of LiAlH₄ (20 mmol) in THF (200 mL) was added a solution of Z-amino acid-DMP (10 mmol) in THF (200 mL) over a period of 1 h keeping the temperature at -15 to -20 °C. After standing for another 1 h at the same temperature, 2 N HCl (12 mL) was added slowly at a temperature below -20 °C under a N₂ stream. After removal of Al(OH)₃ by centrifugation, the solvent was evaporated. The residue was dissolved in Et₂O, washed with H₂O, and then evaporated. To the oily residue was added an ice-cold solution of NaHSO₃ (7-8 mmol) and the mixture was stirred at 5 °C overnight. To the resulting suspension of NaHSO₃ adduct was added EtOAc (200 mL) and KCN aqueous solution (7-8 mmol in 50 mL) and the reaction mixture was stirred (3-4 h) at room temperature. The EtOAc phase was washed with H₂O and then evaporated to give the cyanohydrin as an oil. It was hydrolyzed in dioxane-concentrated HCl (1:1) (100 mL) by reflux (12 h). The hydrolyzate was dried after washing with Et₂O. It was dissolved in H₂O (200 mL); acetone was added (200 mL) and the mixture adjusted to pH 5.5 with NH₄OH. The crystals deposited after standing at 5 °C overnight were filtered and washed with acetone. If crystallization did not occur, the aqueous reaction mixture was adsorbed on a Dowex 50W (H⁺) column, eluted with 2 N NH₄OH, dried, and then washed with acetone. The AHPA analogues thus obtained are summarized in Table V.

Resolution of Stereoisomers of AHPA via Fractional Crystallization of Their Z Derivatives. To an ice-cold solution of 13.66 g (0.07 mol) of (2RS,3R)-AHPA in 70 mL of 1 N NaOH was added 15 mL (0.1 mol) of Z-Cl and 70 mL of 1 N NaOH in three portions over a period of 30 min under vigorous agitation, followed by 1 h of ice cooling and 3 h at room temperature. The pH of the solution was adjusted to 1 with 6 N HCl. The oily material that separated was extracted with EtOAc (2 × 100 mL),

washed with H₂O, dried over MgSO₄, and then evaporated to dryness. The residue was crystallized from EtOAc-petroleum ether to give 8.48 g of crude Z-(2R,3R)-AHPA. Z-(2R,3R)-AHPA was purified by recrystallization from EtOAc-petroleum ether. From the mother liquor, 8.07 g of crude Z-(2S,3R)-AHPA was recovered by precipitation with a small amount of EtOAc and petroleum ether. This material (8.00 g) and 10.35 g of brucine dihydrate were dissolved in EtOAc (300 mL) and MeOH (10 mL) under warming. The solution was filtered and the filtrate was allowed to stand at room temperature overnight. The deposited crystals were filtered and recrystallized twice from EtOAc to give 13.51 g of the brucine salt of Z-(2S,3R)-AHPA: mp 144 °C; $[\alpha]^{21}_{578} +33.8^\circ$ (c 1.1, AcOH). To remove brucine, 13.4 g of the crystals were suspended in 150 mL of EtOAc and 30 mL of 1 N HCl was added. The EtOAc phase was washed with H₂O, dried over MgSO₄, and then evaporated. The residue was crystallized from EtOAc-petroleum ether to give 6.21 g of Z-(2S,3R)-AHPA. In the same way, Z-(2S,3S)-AHPA was isolated from Z-(2RS,3S)-AHPA by crystallization with EtOAc and petroleum ether, and Z-(2R,3S)-AHPA was precipitated as the dehydroabiethylamine salt from the mother liquor fraction: mp 168-169 °C; $[\alpha]^{23}_{578} -31.0^\circ$ (c 1.3, AcOH). Recovery of free Z-(2R,3S)-AHPA was achieved in the same way as for Z-(2S,3R)-AHPA. The Z group was removed by hydrogenation with palladium black in the usual way. The Z-AHPA's and AHPA's thus resolved are summarized in Tables VI and VII.

General Synthetic Procedure for Bestatin and Related Compounds. Z-AHPA or its analogues (Table VIII) (1 mmol), hydroxybenzotriazole (1.2 mmol), amino acid benzyl ester tosylate (1.2 mmol), and Et₃N (1.2 mmol) were dissolved in 10 mL of THF and DCCD (1 mmol) in CH₂Cl₂ (10 mL) was added at 0 °C and allowed to stand overnight. The solvent was evaporated and the residue was extracted with EtOAc. The EtOAc extract was washed with 1 N H₂SO₄, H₂O, 2% NaHCO₃ and H₂O, successively, dried over MgSO₄, and then evaporated. The residue was crystallized from EtOAc-petroleum ether. Recrystallization from the same

Table VIII. Z-AHPA Analogues

Compd	R	Stereochemistry		Yield, %	Mp, °C	[α] ²⁰⁻²⁵ ₅₇₈ in AcOH	Formula	Analyses
		2	3					
		$\begin{array}{c} \text{R}^3 \text{CH} \text{---} \text{CH}^2 \text{COOH} \\ \quad \\ \text{ZHN} \quad \text{OH} \end{array}$						
47a	H	S		79.0	128-129	+4.6	C ₁₁ H ₁₃ O ₅ N	C, H, N
47b	H	R		73.6	126-128	-4.2	C ₁₁ H ₁₃ O ₅ N	H, N; C ^a
48	Me ^b	RS	R	59.2			C ₂₄ H ₃₈ O ₅ N ₂	C, H, N
49	<i>i</i> -Bu ^c	RS	R					
50	Ph	RS	R	57.5			C ₁₇ H ₁₇ O ₅ N	C, H, N
51	<i>o</i> -Cl-Bzl	RS	RS	38.5			C ₁₈ H ₁₈ O ₅ NCI	C, H, N
52	<i>p</i> -Cl-Bzl	RS	RS	57.0			C ₁₈ H ₁₈ O ₅ NCI	H, N; C ^d
53	<i>p</i> -CH ₃ -Bzl ^b	RS	RS	47.8			C ₃₁ H ₄₄ O ₅ N ₂	C, H, N

^a C: calcd, 55.23; found, 55.64. ^b As the dicyclohexylamine salt. ^c Oily. ^d C: calcd, 59.42; found, 59.83.

Table IX. Z-AHPA-Leu-OBzl

Compd	Stereochemistry		Leu	Yield, %	Mp, °C	[α] ²⁰⁻²⁵ ₅₇₈ in AcOH	Analyses ^a
	AHPA						
	2	3					
54a	<i>S</i>	<i>R</i>	<i>S</i>	82.4	122-123	+13.6	C, H, N
54b	<i>S</i>	<i>R</i>	<i>R</i>	84.2	118-119	+43.0	C, H, N
54c	<i>S</i>	<i>S</i>	<i>S</i>	66.5	128	-38.0	C, H, N
54d	<i>S</i>	<i>S</i>	<i>R</i>	67.3	128-129	-11.0	C, H, N
54e	<i>R</i>	<i>R</i>	<i>S</i>	61.6	129.5	+11.2	C, H, N
54f	<i>R</i>	<i>R</i>	<i>R</i>	90.5	128	+36.5	H, N; C ^b
54g	<i>R</i>	<i>S</i>	<i>S</i>	54.9	118-119	-41.9	H, N; C ^c
54h	<i>R</i>	<i>S</i>	<i>R</i>	67.9	122-123	-15.3	C, H, N

^a Calcd for C₃₁H₃₆O₆N₂: C, 69.90. ^b Found: 70.31. ^c Found: 69.38.

Table X. Z-(2S,3R)-AHPA-R-OBzl

Compd	R	Yield, %	Mp, °C	[α] ²⁰⁻²⁵ ₅₇₈ in AcOH	Formula	Analyses
55	Gly ^a					
56	β-Ala	82.3	139-140	+34.3	C ₂₈ H ₃₀ O ₆ N ₂	C, H, N
57	Gaba ^{a,b}					
58	ε-Aca ^c	65.9	79	+32.3	C ₃₁ H ₃₆ O ₆ N ₂	C, H, N
59	L-Pro	73.4	139-140	-10.6	C ₃₀ H ₃₂ O ₆ N ₂	H, N; C ^d
60	L-Lys ^e	79.2	138-139	+24.0	C ₃₉ H ₄₃ O ₈ N ₃	C, H, N
61	L-Glu ^f	94.9	119-120	+28.6	C ₃₇ H ₃₈ O ₈ N ₂	C, H, N
62	L-Gln	36.5	115	+62.3	C ₃₀ H ₃₃ O ₇ N ₃	C, H, N
63	L-Ser ^g	84.2	122	+17.7	C ₃₅ H ₃₅ O ₉ N ₃	H, N; C ^h
64	L-Ala	94.2	127	+13.9	C ₂₈ H ₃₀ O ₆ N ₂	C, H, N
65	L-Val	81.6	97-98	+19.2	C ₃₀ H ₃₄ O ₆ N ₂	C, H, N
66	L-Nva	59.8	114-115	+22.0	C ₃₀ H ₃₄ O ₆ N ₂	C, H, N
67	L-Ile	87.6	100-101	+23.6	C ₃₁ H ₃₆ O ₆ N ₂	C, H, N
68	L-Nle	82.0	114-115	+23.3	C ₃₁ H ₃₆ O ₆ N ₂	H, N; C ⁱ
69	L-Phe	66.8	111-112	+39.6	C ₃₄ H ₃₄ O ₆ N ₂	C, H, N
70	DL-Aoc ^j	76.7			C ₃₃ H ₄₆ O ₆ N ₂	C, H, N

^a Oily. ^b γ-Aminobutyric acid. ^c ε-Aminocaproic acid. ^d C: calcd, 69.75; found, 69.23. ^e Lys(Z). ^f Glu(Obzl). ^g Ser(Bzl)-OBzl(NO₂). ^h C: calcd, 65.51; found, 65.99. ⁱ C: calcd, 69.90; found, 69.48. ^j DL-α-Aminooctanoic acid.

solvent gave analytically pure Z-bestatin benzyl ester or related compounds (Tables IX and X). The protective group was removed by hydrogenation with palladium black in MeOH and H₂O (1:1) for 4-5 h. The resulting bestatin or related compounds were crystallized from MeOH-EtOAc (Tables I-III).

Boc-(2S,3R)-AHPA-L-Met-OMe (72). The dicyclohexylamine salt of Boc-(2S,3R)-AHPA (71) was obtained in the usual manner in 77% yield using *tert*-butyl (S)-4,6-dimethylpyrimidin-2-ylthiocarbonate;⁵ mp 158-159 °C; [α]²⁵₅₇₈ +51.9° (c 0.89, AcOH). The free oily Boc-(2S,3R)-AHPA, which was obtained by extraction with EtOAc from acidic solution of the dicyclohexylamine salt (952 mg, 2 mmol), and L-methionine methyl ester hydrochloride (440 mg, 2.2 mmol) were suspended in THF (20 mL) and neutralized with Et₃N (0.308 mL). To the resulting solution was added DCCD (412 mg) at 0 °C followed by 4 h of agitation at room temperature. The coupling product, which was extracted in the aforementioned manner, was dissolved in EtOAc-C₆H₆ (2:5) and chromatographed on a silica gel 60 (Merck) column (1.5 × 40 cm) developed with the same solvent. The fractions containing the

desired material were collected and evaporated to give solid material. Recrystallization from Et₂O-petroleum ether gave 380 mg (42.8%) of the title compound: mp 118 °C; [α]²⁴₅₇₈ +51.4° (c 0.56, AcOH).

Boc-(2S,3R)-AHPA-L-Met (73). 72 (280 mg, 0.63 mmol) was treated with 0.65 mL of 1 N NaOH in MeOH (10 mL) for 1 h at room temperature. The solvent was removed and the residue was dissolved in H₂O and adjusted to pH 2 with 1 N H₂SO₄. The precipitate was extracted with EtOAc, washed with H₂O, dried over MgSO₄, and then evaporated. The residue was crystallized from EtOAc-petroleum ether. Recrystallization from the same solvent gave 203 mg (74%) of 73: mp 116 °C dec; [α]²²₅₇₈ +44.1° (c 0.54, AcOH).

(2S,3R)-AHPA-L-Met (11). 73 (150 mg, 0.35 mmol) was dissolved in 50% aqueous TFA (10 mL) and 0.2 mL of thioglycolic acid was added. The mixture was agitated for 1 h at room temperature and then evaporated. The oily residue was dissolved in H₂O, adsorbed on a Dowex 50W (H⁺) column (1 × 7 cm), eluted with 2 N NH₄OH, and dried. The residue was reprecipitated from

H₂O-CH₃COCH₃ (1:1) to give 87 mg (55%) of 11 (Table II).

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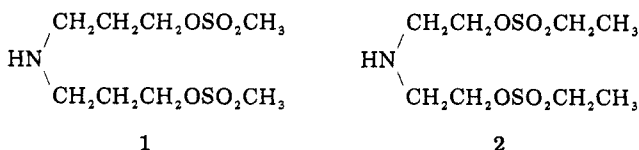
Potential Antitumor Agents. 21. Dialkanolamine Dialkanesulfonic Esters

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Homologous dialkanesulfonic esters of 2,2'-iminodiethanol, 3,3'-iminodi-1-propanol, *N*-(2-hydroxyethyl)-6-amino-1-hexanol, and *N*-(3-hydroxypropyl)-6-amino-1-hexanol were prepared via the *N*-trityl derivatives and screened for L1210 activity. For each active agent significant increases in life-span in L1210 tests (ILS) were correlated by linear least-squares regression with the corresponding log doses. The maximum ILS, taken from this regression line at the determined LD₁₀, was used as a quantitative measure of antileukemic effectiveness. Within each homologous series log ILS_{max} could be correlated with a binomial expression in lipophilic-hydrophilic balance, as measured by *R*_m values, but all active members from the four series could not be successfully included in a single correlation. By modification of *R*_m values with an ionization factor, log (H⁺/H⁺ + *K*_a), all active compounds could then be included in a significant correlation equation.

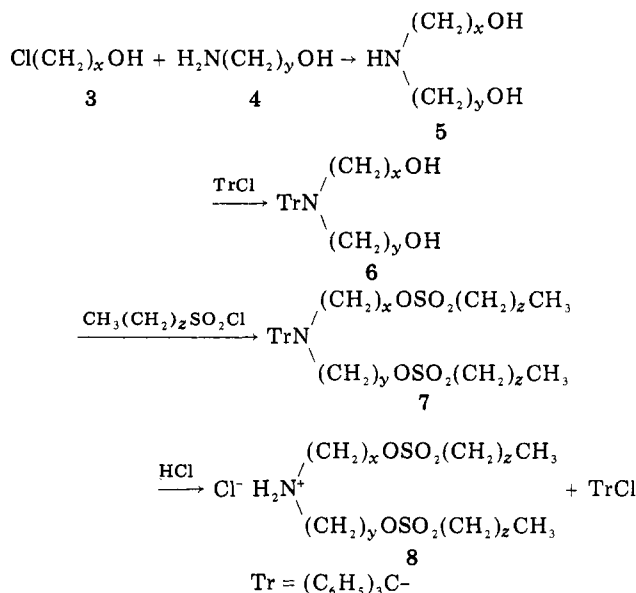
A structurally simple antitumor agent containing an acylable amine function and having relatively high activity was required as the core molecule for projected latentiation studies.¹ From published data² 3,3'-iminodi-1-propanol dimethanesulfonate (1) appeared a suitable candidate. The recorded² antitumor activity (L1210) for this compound (1) is high in comparison with that found with nitrogen mustard, *N*-methyl-2,2'-dichlorodiethylamine (see Table I for comparative L1210 test data for these two agents). Our past experience has been that in many cases such differences in biologic activity result not from differing chemical structures per se but rather from the divergent physicochemical properties of the agents.^{1,3,4} Usually drug lipophilic-hydrophilic balance has been found to be the dominant physicochemical parameter affecting biologic activity. To more rigorously assess the contribution of drug structural features to biologic activity, we have employed the simplifying device of conducting all agent comparisons at the equivalent of equilipophilicity.^{1,3} Adopting this strategy, the biologic activity of molecules such as 1 and nitrogen mustard, these having different lipophilic characters (e.g., Σπ values), would not be compared but rather, for example, those of 1 and the



diethanesulfonic ester of 2,2'-iminodiethanol 2—these two molecules having comparable Σπ values. Additionally, assessment of the maximum level of activity attainable in an agent series requires a knowledge of the optimum drug lipophilic-hydrophilic balance. Screening data from lower members of the homologous alkanesulfonic esters of 2,2'-iminodiethanol might then assist in answering the queries: (a) are there specific structural features of 1 which provide increased biologic selectivity, and (b) what agent lipophilic-hydrophilic balance is necessary for maximum experimental antileukemic activity?

Synthetic methods allowing convenient preparation of homologous series of alkanolamine alkanesulfonic esters,

Scheme I



antitumor (L1210) screening data, and quantitative structure-biologic activity relationships for these compounds form the basis of this publication.

Chemistry. Required alkanolamines 5 were prepared from chlorohydrin (3) and amine precursors 4 in alkaline media (Scheme I). Masking of alkanolamine nitrogen by tritylation, as in 6, provided virtually nonbasic, crystalline, lipophilic derivatives—contrasting with the basic, viscous, hydrophilic precursors. Reaction of the *N*-tritylalkanolamines 6 with alkanesulfonyl chlorides in pyridine provided high yields (>90%) of the sulfonate esters 7. Most lower members of the homologous *N*-tritylalkanesulfonic esters 7 were crystalline solids but the higher homologues were not obtained crystalline. The lipophilic nature conferred by the trityl function permits solutions of these noncrystalline materials in nonpolar solvents to be freed of excess reagents that could interfere in the final demasking step (7 → 8). Treatment of dioxane solutions of the sulfonate esters 7 with dry HCl precipitated the agent