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

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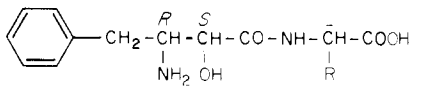
Stereoisomers and analogues of bestatin, [(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine, were synthesized and tested for aminopeptidase B and leucine aminopeptidase inhibiting activity. Among the eight stereoisomers, the 2*S* stereoisomers exhibited strong activity. In a series of compounds 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}.



R = $-CH_2CH(CH_3)_2$

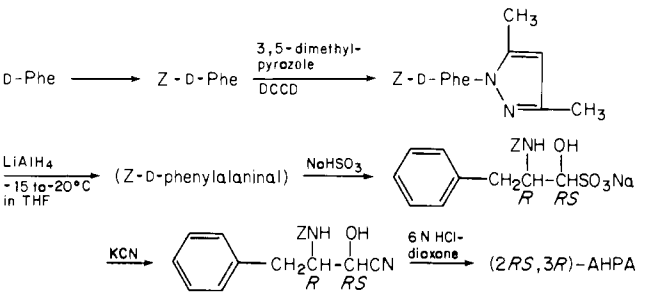
Bestatin is [(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine.[2] (2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutanoic acid [abbreviated as (2*S*,3*R*)-AHPA] is a new amino acid. We have synthesized stereoisomers of bestatin and related compounds and studied structure–activity relationships.

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-phenylalaninal, 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 threo 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

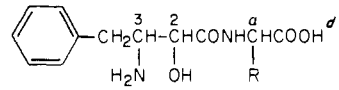


The protecting group was removed by hydrogenation with palladium black to yield bestatin analogues. For the preparation of AHPA-methionine, *N*-*t*-Boc-AHPA was coupled with methionine methyl ester followed by alkali and trifluoroacetic acid treatments to remove the protecting groups. *p*-Nitrobestatin 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 μg/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 μg/mL and the amide of bestatin had only very weak activity (IC_{50} = 35 μg/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 μg/mL) and its amide and *N*-benzyloxycarbonyl derivative did not inhibit at 250 μg/mL.

Bryce and Rabin[3] 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

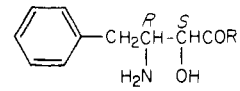
Table I. Bestatin and Its Stereoisomers



Compd	Stereochemistry 2	3	α	Yield, %	R_f[a]	$[\alpha]^{20-25}_{578}$ in AcOH	Formula	Analyses	Act.,[b] IC_{50}, μg/ml AP-B	LAP
1a	*S*	*R*	*S* (=L)	87.9	0.48	−23.5	$C_{16}H_{24}O_4N_2$	C, H, N	0.05	0.01
1b	*S*	*R*	*R* (=D)	57.6	0.49	+0.9	$C_{16}H_{24}O_4N_2$	C, H, N	0.56	3.4
1c	*S*	*S*	*S*	93.4	0.37	−61.3	$C_{16}H_{24}O_4N_2$	C, H, N	1.25	0.07
1d	*S*	*S*	*R*	80.5	0.52	−31.4	$C_{16}H_{24}O_4N_2$	C, H, N	0.05	2.7
1e	*R*	*R*	*S*	63.8	0.52	+30.3	$C_{16}H_{24}O_4N_2$	C, H, N	100	7.5
1f	*R*	*R*	*R*	64.3	0.37	+60.2	$C_{16}H_{24}O_4N_2$	H, N; C[c]	>250	>250
1g	*R*	*S*	*S*	73.5	0.49	−1.4	$C_{16}H_{24}O_4N_2$	C, H, N	>250	>250
1h	*R*	*S*	*R*	58.9	0.48	+22.7	$C_{16}H_{24}O_4N_2$	C, H, N	135	>250

[a] Developing solvent, BuOH-AcOH-H_2O (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 = $-CH_2CH(CH_3)_2$.

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



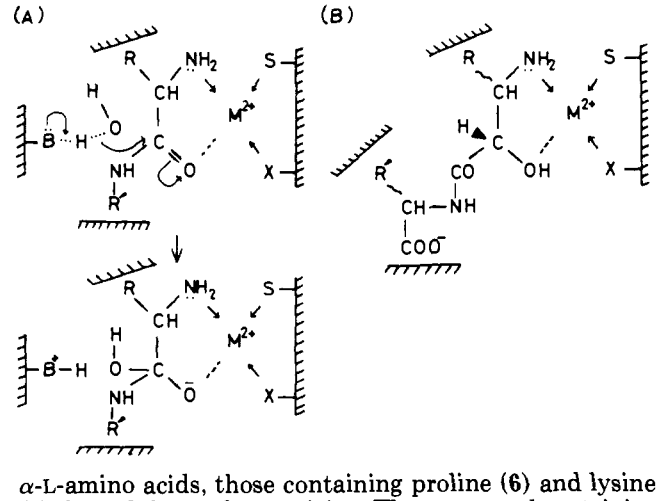
Compd	R	Yield, %	R_f[a]	$[\alpha]^{20-25}_{578}$ in AcOH	Formula	Analyses	Act.[b] (AP-B), IC_{50}, μg/ml
2	Gly	46.5[c]	0.26	−7.4	$C_{12}H_{16}O_4N_2 \cdot CH_3OH$	H, N; C[d]	21.5
3	β-Ala	67.7	0.34	−2.3	$C_{13}H_{18}O_4N_2$	C, H, N	37.5
4	Gaba[e]	10.5[c]	0.35	−14.5	$C_{14}H_{20}O_4N_2 \cdot 0.5H_2O$	C, H, N	110
5	ε-Aca[f]	78.0	0.48	−16.8	$C_{16}H_{24}O_4N_2$	C, H, N	110
6	L-Pro	74.1	0.25	−54.1	$C_{15}H_{20}O_4N_2 \cdot H_2O$	C, H, N	>250
7	L-Lys	88.3	0.06	−2.0	$C_{16}H_{25}O_4N_3 \cdot 1.5CH_3OH \cdot H_2O$	C, H, N	>250
8	L-Glu	93.3	0.27	−19.4	$C_{15}H_{20}O_6N_2$	C, H, N	25
9	L-Gln	51.7	0.17	−9.9	$C_{15}H_{21}O_5N_3 \cdot H_2O$	C, H, N	1.2
10	L-Ser	66.7	0.20	−9.0	$C_{13}H_{18}O_5N_2 \cdot H_2O$	H, N; C[g]	0.7
11	L-Met	55.0	0.37	−20.4	$C_{15}H_{22}O_4N_2S \cdot 0.5H_2O$	C, N; H[h]	0.22
12	L-Ala	79.5	0.21	−30.3	$C_{13}H_{18}O_4N_2$	C, H, N	0.27
13	L-Val	90.5	0.42	−1.4	$C_{15}H_{22}O_4N_2$	C, H, N	0.55
14	L-Nva	77.6	0.41	−14.5	$C_{15}H_{22}O_4N_2 \cdot 0.5H_2O$	C, H, N	0.17
15	L-Ile	73.7	0.48	+6.4	$C_{16}H_{24}O_4N_2$	C, H, N	0.04
16	L-Nle	79.0	0.47	−8.9	$C_{16}H_{24}O_4N_2 \cdot H_2O$	C, H, N	0.13
17	L-Phe	50.1	0.44	+0.4	$C_{19}H_{22}O_4N_2 \cdot CH_3OH$	C, H, N	4.2
18	DL-Aoc[i]	49.3	0.58	−24.9	$C_{18}H_{28}O_4N_2$	C, H, N	3.1
19	Isoamylamine hydrochloride	67.0	0.65	−18.9	$C_{15}H_{25}O_2N_2Cl$	C, H, N	170
20	L-Leu-Gly	51.5	0.34	−27.8	$C_{18}H_{27}O_5N_3$	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-Aminooctanoic acid.

I indicate that the configuration at C_2, but not that at C_3, 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, (2*S*,3*S*)-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 C_2 of AHPA is a requirement for enzyme inhibition suggests that in the enzymic reaction the hydration of the carbonyl group is stereospecific.

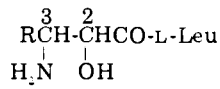
Bestatin-related compounds in which the L-leucine moiety is substituted by other amino acids, a peptide, or an amine are shown in Table II. Among those containing α-, β-, γ-, and ε-amino acids, the compound in which the amino group was α to the carboxyl group (**2**) showed the strongest activity. It 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 Rabin[3] (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

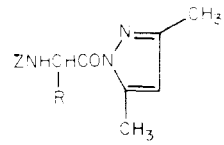
Table III. Substitution for the Benzyl Group of Bestatin



Compd	R	Stereochemistry 2	Stereochemistry 3	Yield, %	R_f[a]	$[\alpha]^{20-25}_{578}$ in AcOH	Formula	Analyses	Act.[b] (AP-B), IC_{50}, μg
21a	H	*S*		78.0	0.13	−57.9	$C_9H_{18}O_4N_2$	H, N; C[c]	140
21b	H	*R*		92.0	0.13	+8.2	$C_9H_{18}O_4N_2$	C, H, N	>250
22	Me	*RS*	*R*	71.6[d]	0.23, 0.27[e]	−28.7	$C_{10}H_{20}O_4N_2$	C, H, N	16
23	*i*-Bu	*RS*	*R*	28.4[d]	0.43, 0.49[e]	−26.6	$C_{13}H_{26}O_4N_2$	C, H, N	12
24	c-C_6H_{10}-Me	*S*	*R*	95.3[f]	0.47	−30.5	$C_{16}H_{30}O_4N_2$	C, H, N	1.1
25	Ph	*RS*	*R*	81.7	0.38, 0.41[e]	−2.4	$C_{15}H_{22}O_4N_2$	C, H, N	56
26	*p*-NO_2-Bzl	*S*	*R*	67.0[f]	0.58	+5.2	$C_{16}H_{23}O_6N_3$	C, H, N	0.01
27	*p*-NH_2-Bzl	*S*	*R*	90.5[g]	0.25	−23.2	$C_{16}H_{25}O_4N_3$	H, N; C[h]	0.10
28	*o*-Cl-Bzl	*RS*	*RS*	61.9	0.46	−20.8	$C_{16}H_{23}O_4N_2Cl$	C, H, N	0.48
29	*p*-Cl-Bzl	*RS*	*RS*	71.9	0.42, 0.52[e]	−11.3	$C_{16}H_{23}O_4N_2Cl$	C, N; H[i]	0.07
30	*p*-CH_3-Bzl	*RS*	*RS*	51.5[d]	0.53, 0.58[e]	−7.2	$C_{17}H_{26}O_4N_2$	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	Stereochemistry	Yield, %	Mp, °C	$[\alpha]^{20-25}_{578}$ in AcOH	Formula	Analyses
31a	Bzl	*R*	93.5	139	−73.0	$C_{22}H_{23}O_3N_3$	C, H, N
31b	Bzl	*S*	92.8	142	+73.9	$C_{22}H_{23}O_3N_3$	C, H, N
32	Me	*R*	82.4	121-122	−11.1	$C_{16}H_{19}O_3N_3$	C, H; N[a]
33	*i*-Bu	*R*	60.4	60-61	−12.9	$C_{19}H_{25}O_3N_3$	C, H, N
34	Ph[b]	*R*					
35	*o*-Cl-Bzl	*RS*	90.0			$C_{22}H_{22}O_3N_3Cl$	C, H, N
36	*p*-Cl-Bzl	*RS*	73.1			$C_{22}H_{22}O_3N_3Cl$	C, H, N
37	*p*-CH_3-Bzl	*RS*	61.7			$C_{23}H_{25}O_3N_3$	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.

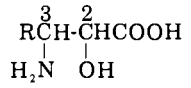
The activity of bestatin-related compounds in which the benzyl group of bestatin is substituted by hydrogen, alkyl, phenyl, and substituted benzyl groups is shown in Table III. (*S*)-Isoseryl-L-leucine (**21a**) showed weak activity and the *R* isomer **21b** was inactive. This also indicates the importance of the *S* configuration at C_2 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 (IC_{50}). 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 F_{254} 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 $CHCl_3$ (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 2	Stereochemistry 3	Yield, %	R_f[a]	Formula	Analyses
38	Me	*RS*	*R*	72.8	0	$C_4H_9O_3N$	C, H, N
39	*i*-Bu	*RS*	*R*	64.2	0.18	$C_7H_{15}O_3N$	C, H, N
40	Ph	*RS*	*R*	8.8	0.13, 0.17[b]	$C_9H_{11}O_3N$	H, N; C[c]
41a	Bzl	*RS*	*R*	55.2	0.23, 0.26[b]	$C_{10}H_{13}O_3N$	H, N; C[d]
41b	Bzl	*RS*	*S*	31.7	0.23, 0.26[b]	$C_{10}H_{13}O_3N$	C, H, N
42	*o*-Cl-Bzl	*RS*	*RS*	58.3	0.25, 0.29[b]	$C_{10}H_{12}O_3NCl$	C, H, N
43	*p*-Cl-Bzl	*RS*	*RS*	37.0	0.25	$C_{10}H_{12}O_3NCl$	C, H, N
44	*p*-CH_3-Bzl	*RS*	*RS*	5.3	0.20	$C_{11}H_{15}O_3N$	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 2	Stereochemistry 3	Yield, %	Mp, °C	$[\alpha]^{20-25}_{578}$ in AcOH	Formula	Analyses
45a	*S*	*R*	67.0	154-155	+83.5	$C_{18}H_{19}O_5N$	C, H, N
45b	*R*	*R*	47.6	175-176	+5.7	$C_{18}H_{19}O_5N$	C, H, N
45c	*S*	*S*	41.3	175-176	-6.3	$C_{18}H_{19}O_5N$	H, N; C[a]
45d	*R*	*S*	36.1	154-155	-84.2	$C_{18}H_{19}O_5N$	C, H, N

[a] C: calcd, 65.64; found, 65.13.

Table VII. AHPA

Compd	Stereochemistry 2	Stereochemistry 3	Yield	$[\alpha]^{20-25}_{578}$ in 1 N HCl	Formula	Analyses
46a	*S*	*R*	Quantitative	+29.5	$C_{10}H_{13}O_3N \cdot {}^1/_3H_2O$	C, H, N
46b	*R*	*R*	Quantitative	+5.2	$C_{10}H_{13}O_3N$	H, N; C[a]
46c	*S*	*S*	Quantitative	-5.6	$C_{10}H_{13}O_3N$	H, N; C[b]
46d	*R*	*S*	Quantitative	-31.0	$C_{10}H_{13}O_3N \cdot {}^1/_3H_2O$	C, H, N

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

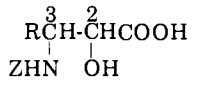
and washed with 1 N HCl and H_2O. The solvent phase was dried with $MgSO_4$ and evaporated to give a solid residue which was crystallized from EtOAc–petroleum ether. The Z-amino acid–DMP products thus obtained are summarized in Table IV.

General Synthetic Procedure for AHPA and Its Analogues. To a suspension of $LiAlH_4$ (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 N_2 stream. After removal of $Al(OH)_3$ by centrifugation, the solvent was evaporated. The residue was dissolved in Et_2O, washed with H_2O, and then evaporated. To the oily residue was added an ice-cold solution of $NaHSO_3$ (7–8 mmol) and the mixture was stirred at 5 °C overnight. To the resulting suspension of $NaHSO_3$ 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 H_2O 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 Et_2O. It was dissolved in H_2O (200 mL); acetone was added (200 mL) and the mixture adjusted to pH 5.5 with NH_4OH. 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 NH_4OH, 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 (2*RS*,3*R*)-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 H_2O, dried over $MgSO_4$, and then evaporated to dryness. The residue was crystallized from EtOAc–petroleum ether to give 8.48 g of crude Z-(2*R*,3*R*)-AHPA. Z-(2*R*,3*R*)-AHPA was purified by recrystallization from EtOAc–petroleum ether. From the mother liquor, 8.07 g of crude Z-(2*S*,3*R*)-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-(2*S*,3*R*)-AHPA: mp 144 °C; $[\alpha]^{21}_{578}$ +33.8° (*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 H_2O, dried over $MgSO_4$, and then evaporated. The residue was crystallized from EtOAc–petroleum ether to give 6.21 g of Z-(2*S*,3*R*)-AHPA. In the same way, Z-(2*S*,3*S*)-AHPA was isolated from Z-(2*RS*,3*S*)-AHPA by crystallization with EtOAc and petroleum ether, and Z-(2*R*,3*S*)-AHPA was precipitated as the dehydroabiethylamine salt from the mother liquor fraction: mp 168–169 °C; $[\alpha]^{23}_{578}$ −31.0° (*c* 1.3, AcOH). Recovery of free Z-(2*R*,3*S*)-AHPA was achieved in the same way as for Z-(2*S*,3*R*)-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 Et_2N (1.2 mmol) were dissolved in 10 mL of THF and DCCD (1 mmol) in CH_2Cl_2 (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 H_2SO_4, H_2O, 2% $NaHCO_3$ and H_2O, successively, dried over $MgSO_4$, and then evaporated. The residue was crystallized from EtOAc–petroleum ether. Recrystallization from the same

Table VIII. Z-AHPA Analogues



Compd	R	Stereochemistry 2	Stereochemistry 3	Yield, %	Mp, °C	$[\alpha]^{20-25}_{578}$ in AcOH	Formula	Analyses
47a	H	*S*		79.0	128-129	+4.6	$C_{11}H_{13}O_5N$	C, H, N
47b	H	*R*		73.6	126-128	−4.2	$C_{11}H_{13}O_5N$	H, N; C[a]
48	Me[b]	*RS*	*R*	59.2			$C_{24}H_{38}O_5N_2$	C, H, N
49	*i*-Bu[c]	*RS*	*R*					
50	Ph	*RS*	*R*	57.5			$C_{17}H_{17}O_5N$	C, H, N
51	*o*-Cl-Bzl	*RS*	*RS*	38.5			$C_{18}H_{18}O_5NCl$	C, H, N
52	*p*-Cl-Bzl	*RS*	*RS*	57.0			$C_{18}H_{18}O_5NCl$	H, N; C[d]
53	*p*-CH_3-Bzl[b]	*RS*	*RS*	47.8			$C_{31}H_{44}O_5N_2$	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 AHPA 2	Stereochemistry AHPA 3	Leu	Yield, %	Mp, °C	$[\alpha]^{20-25}_{578}$ in AcOH	Analyses[a]
54a	*S*	*R*	*S*	82.4	122-123	+13.6	C, H, N
54b	*S*	*R*	*R*	84.2	118-119	+43.0	C, H, N
54c	*S*	*S*	*S*	66.5	128	−38.0	C, H, N
54d	*S*	*S*	*R*	67.3	128-129	−11.0	C, H, N
54e	*R*	*R*	*S*	61.6	129.5	+11.2	C, H, N
54f	*R*	*R*	*R*	90.5	128	+36.5	H, N; C[b]
54g	*R*	*S*	*S*	54.9	118-119	−41.9	H, N; C[c]
54h	*R*	*S*	*R*	67.9	122-123	−15.3	C, H, N

[a] Calcd for $C_{31}H_{36}O_6N_2$: C, 69.90. [b] Found: 70.31. [c] Found: 69.38.

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

Compd	R	Yield, %	Mp, °C	$[\alpha]^{20-25}_{578}$ in AcOH	Formula	Analyses
55	Gly[a]					
56	β-Ala	82.3	139-140	+34.3	$C_{28}H_{30}O_6N_2$	C, H, N
57	Gaba[a,b]					
58	ε-Aca[c]	65.9	79	+32.3	$C_{31}H_{36}O_6N_2$	C, H, N
59	L-Pro	73.4	139-140	−10.6	$C_{30}H_{32}O_6N_2$	H, N; C[d]
60	L-Lys[e]	79.2	138-139	+24.0	$C_{39}H_{43}O_8N_3$	C, H, N
61	L-Glu[f]	94.9	119-120	+28.6	$C_{37}H_{38}O_8N_2$	C, H, N
62	L-Gln	36.5	115	+62.3	$C_{30}H_{33}O_7N_3$	C, H, N
63	L-Ser[g]	84.2	122	+17.7	$C_{35}H_{35}O_9N_3$	H, N; C[h]
64	L-Ala	94.2	127	+13.9	$C_{28}H_{30}O_6N_2$	C, H, N
65	L-Val	81.6	97-98	+19.2	$C_{30}H_{34}O_6N_2$	C, H, N
66	L-Nva	59.8	114-115	+22.0	$C_{30}H_{34}O_6N_2$	C, H, N
67	L-Ile	87.6	100-101	+23.6	$C_{31}H_{36}O_6N_2$	C, H, N
68	L-Nle	82.0	114-115	+23.3	$C_{31}H_{36}O_6N_2$	H, N; C[i]
69	L-Phe	66.8	111-112	+39.6	$C_{34}H_{34}O_6N_2$	C, H, N
70	DL-Aoc[j]	76.7			$C_{33}H_{46}O_6N_2$	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(NO_2). [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 H_2O (1:1) for 4–5 h. The resulting bestatin or related compounds were crystallized from MeOH–EtOAc (Tables I–III).

Boc-(2*S*,3*R*)-AHPA-L-Met-OMe (72). The dicyclohexylamine salt of Boc-(2*S*,3*R*)-AHPA (71) was obtained in the usual manner in 77% yield using *tert*-butyl (*S*)-4,6-dimethylpyrimidin-2-yl-thiocarbonate:[5] mp 158–159 °C; $[\alpha]^{25}_{578}$ +51.9° (*c* 0.89, AcOH). The free oily Boc-(2*S*,3*R*)-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 Et_3N (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–C_6H_6 (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 Et_2O–petroleum ether gave 380 mg (42.8%) of the title compound: mp 118 °C; $[\alpha]^{24}_{578}$ +51.4° (*c* 0.56, AcOH).

Boc-(2*S*,3*R*)-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 H_2O and adjusted to pH 2 with 1 N H_2SO_4. The precipitate was extracted with EtOAc, washed with H_2O, dried over $MgSO_4$, 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; $[\alpha]^{22}_{578}$ +44.1° (*c* 0.54, AcOH).

(2*S*,3*R*)-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 H_2O, adsorbed on a Dowex 50W (H^+) column (1 × 7 cm), eluted with 2 N NH_4OH, and dried. The residue was reprecipitated from

H_2O–CH_3COCH_3 (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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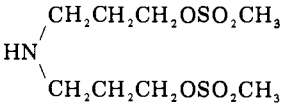
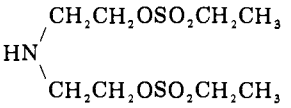
Homologous dialkanesulfonic esters of 2,2′-iminodiethanol, 3,3′-iminodi-1-propanol, *N*-(2-hydroxyethyl)-6-amino-1-hexanol, and *N*-(3-hydroxypropyl)-6-amino-1-hexanol were prepared via the *N*-trityl derivatives and screened for L1210 activity. For each active agent significant increases in life-span 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 LD_{10}, 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 data[2] 3,3′-iminodi-1-propanol dimethanesulfonate (**1**) appeared a suitable candidate. The recorded[2] 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., $\Sigma\pi$ values), would not be compared but rather, for example, those of **1** and the



1

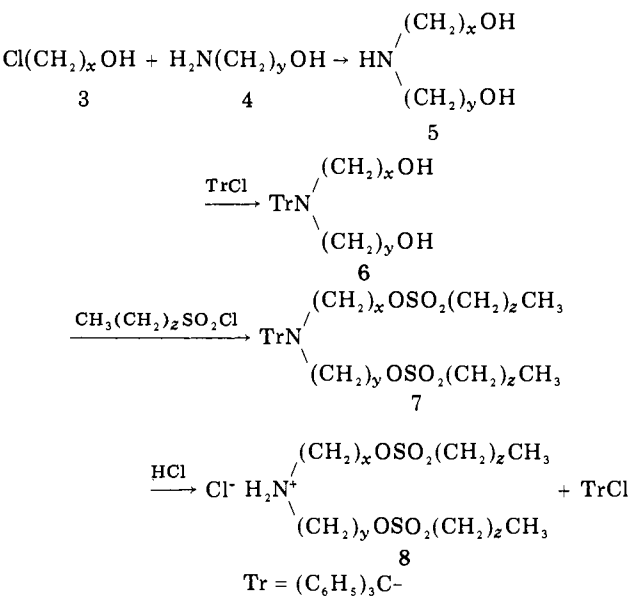


2

diethanesulfonic ester of 2,2′-iminodiethanol **2**—these two molecules having comparable $\Sigma\pi$ 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