Aminobenzoic Acid Diuretics. 6.[1] 4-Substituted 3-Alkylthio-5-sulfamoylbenzoic and 5-Sulfamoylthiosalicylic Acids

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Certain 4-substituted 3-alkylthio- and 3-alkylsulfonyl-5-sulfamoylbenzoic acids and some corresponding 5-sulfamoylthiosalicylic acids have been synthesized. The results of the diuretic screening in dogs are summarized and compared with those of bumetanide and 3-*n*-butylamino-4-chloro-5-sulfamoylbenzoic acid. In contrast to the earlier described aminobenzoic acid diuretics where high potency and high-ceiling diuretic and saluretic activity were demonstrated for both the 3-aminobenzoic acid series and the anthranilic acid series, comparable potency could be shown only for 3-mercapto-5-sulfamoylbenzoic acid derivatives. Some of the thiosalicyclic acid derivatives were found to be moderately active. The sulfonyl compounds were completely devoid of diuretic activity. For 3-*n*-butylthio-4-phenoxy-5-sulfamoylbenzoic acid (**33**) a dose–response and diuretic potency almost similar to that of bumetanide are demonstrated.

Previous papers of this series have dealt with the structural features required for potent high-ceiling diuretic activity of 4-substituted 3-amino-5-sulfamoylbenzoic and 5-sulfamoylanthranilic acid derivatives.[1-5],† The importance of steric parameters of substituents at the 4 position and the influence of different alkylamino side chains on the diuretic potency have been elucidated.[1] In line with our interest in the structure–activity relationship, we decided to examine whether the amino function was essential for the diuretic activity. Our investigation comprises the title compounds **29–48** and **71–79** as sulfur analogs of aminobenzoic acid diuretics. Furthermore, we extended our study to some of the corresponding sulfonyl compounds **49–53**.

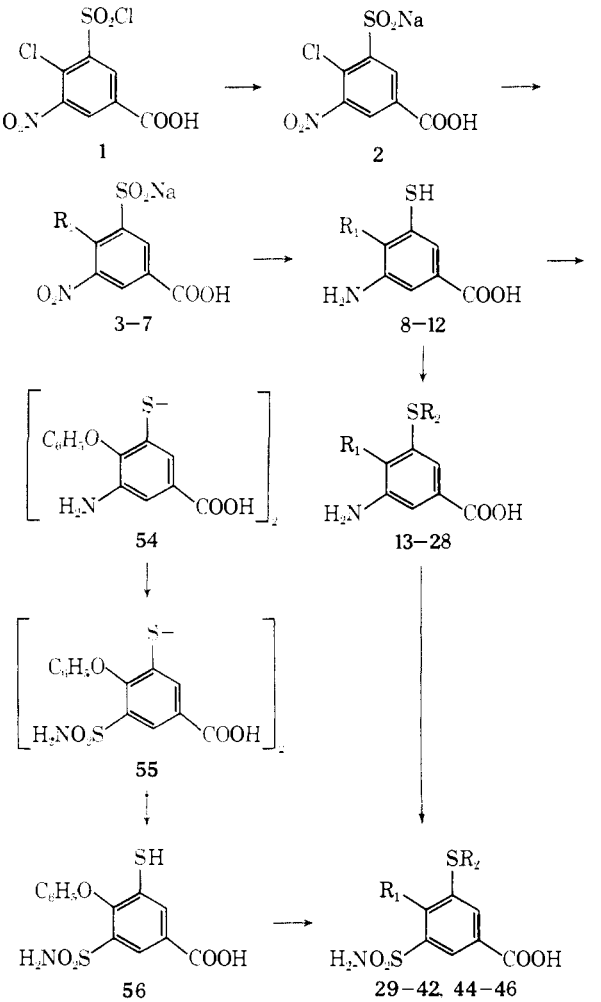
Chemistry. The synthesis of the 4-substituted 3-mercapto-5-sulfamoylbenzoic acid derivatives was mostly achieved as outlined in Scheme I and is detailed in the Experimental Section. Reduction of the monosodium salts of the carboxynitrobenzenesulfinic acids **3–7** (Table I) provided the 4-R_1-5-amino-3-mercaptobenzoic acids **8–12** (Table II) which generally were partially alkylated to the 4-R_1-3-R_2S-5-aminobenzoic acids **13–28** (Table II). Meerwein reaction and subsequent amidation of the resulting sulfochlorides gave the 4-R_1-3-R_2S-5-sulfamoylbenzoic acids **29–39, 42**, and **44–46** (Table III). In the alternative route providing **40** and **41** *via* the disulfides **54** and **55**, the *S*-alkylation was performed as the final step.

3-*n*-Butylsulfonyl-4-phenoxy-5-sulfamoylbenzoic acid (**51**) was obtained by oxidation of the *n*-butylthiobenzoic acid **33**, while the corresponding 4-phenylthiobenzoic acid **52** and 3-benzylsulfonyl-4-phenylthio-5-sulfamoylbenzoic acid (**53**) were achieved as indicated in Scheme II. The sequence chosen made, furthermore, the 3-R_2S-4-chloro-5-sulfamoylbenzoic acids **47** and **48** and the corresponding sulfonyl compounds **49** and **50** available. The chlorobenzoic acid **47** was used for the preparation of the corresponding phenylthiobenzoic acid **43** (Table III) since the Meerwein reaction with the amine **25** following the general route proceeded unsatisfactorily.

The thiosalicylic acid derivatives **71–79** (Table IV) were provided as given in Scheme III. For further details see the Experimental Section.

Diuretic Effect and Structure–Activity Relationship. The 4-substituted 3-alkylthio-5-sulfamoylbenzoic acids **29–48**, the sulfonyl compounds **49–53**, and the 4-substituted 5-sulfamoylthiosalicylic acid derivatives **71–79** prepared in this study were screened in dogs for their diuretic properties after intraveneous and in some cases after oral administration. The urinary volume and electrolyte excretion from the 3-hr test period (Table III) revealed that many compounds of the 3-alkylthio-5-sulfamoylbenzoic acid series exhibit excellent diuretic potency, when compared with 3-*n*-butylamino-4-phenoxy-5-sulfamoylbenzoic acid (bumetanide). The onset of diuresis was observed within the first hour after injection and became negligible after 3 hr with the exception of potent compounds at higher dosage. The dependence of the diuretic potency on the alkyl in the 3-alkylthio side chain cannot clearly be distinguished from that on the alkyl in the 3-alkylamino side chain of the corresponding 4-substituted 3-amino-5-sulfamoylbenzoic acid series.[3] Furthermore, the reported[6] high-ceiling dose–response and diuretic potency of bumetanide after intraveneous and oral administration in the

Scheme I



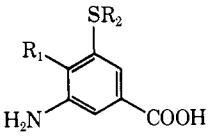
† In ref 2 the term metanilic acid has been used erroneously for 3-aminobenzoic acid throughout.

Table I. Physical Properties of Compounds **3–7**

No.	R_1	Method[a]	Mp, °C	Recrystn solvent[b]	Yield, %[c]	Formula[d]
3	OC_6H_5	A	223 dec	H_2O	58	$C_{13}H_8NNaO_7S \cdot 3H_2O$
4	OC_6H_4, 4-OMe	B	208 dec	H_2O	9[e]	$C_{14}H_{10}NNaO_8S \cdot 3H_2O$[f]
5	SC_6H_5	C	237 dec	H_2O	37	$C_{13}H_8NNaO_6S_2 \cdot H_2O$
6	SC_6H_4, 2-Me	D	225–235 dec	H_2O[g]	19	$C_{14}H_{10}NNaO_6S_2 \cdot 1.75H_2O$
7	SC_6H_4, 4-Me	C	222–224 dec	H_2O	52	$C_{14}H_{10}NNaO_6S_2 \cdot 2H_2O$

[a]The letters relate to the general procedure given in the Experimental Section. [b]Several recrystallizations were usually performed if necessary while treating with decolorizing C. [c]The yield of the analytically pure compounds is given, and in most cases no attempts were made to optimalize the yield. The compounds were dried in air. [d]The compounds were analyzed for C, H, N, and H_2O. Analytical results are within 0.4% of the theoretical values. [e]45% after one recrystallization. [f]H_2O: calcd, 12.59; found, 12.12. [g]The pH was adjusted to 4 during recrystallization; otherwise impure material was obtained probably due to partial precipitation of free sulfinic acid.

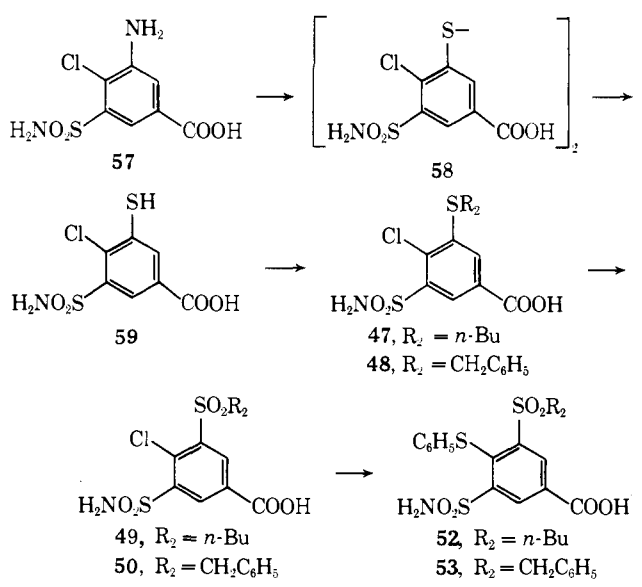
Table II. Physical Properties of



No.	R_1	R_2	Method[a]	Mp, °C	Recrystn solvent[b]	Yield, %[c]	Formula[d]
8	OC_6H_5	H	E	202	Aq EtOH	47	$C_{13}H_{11}NO_3S$
9	OC_6H_4, 4-OMe	H	E	184–185	Aq EtOH	48	$C_{14}H_{13}NO_4S$
10	SC_6H_5	H	E	172–174	Me_2CO–H_2O	27	$C_{13}H_{11}NO_2S_2$
11	SC_6H_4, 2-Me	H	E	205–206	Aq EtOH	50	$C_{14}H_{13}NO_2S_2$
12	SC_6H_4, 4-Me	H	E	186–188	Aq EtOH	24	$C_{14}H_{13}NO_2S_2$
13	OC_6H_5	Et	F	154–155	Aq EtOH	68	$C_{15}H_{15}NO_3S \cdot 0.5H_2O$
14	OC_6H_5	*n*-Pr	G	136–138	Aq EtOH	33	$C_{16}H_{17}NO_3S$
15	OC_6H_5	$CH_2CH{=}CH_2$	H	142–143	Aq EtOH	32	$C_{16}H_{15}NO_3S$[e]
16	OC_6H_5	$CH_2C{\equiv}CH$	I	167–168	Aq EtOH	53	$C_{16}H_{13}NO_3S$
17	OC_6H_5	*n*-Bu	J	131–132	EtOH–H_2O	76	$C_{17}H_{19}NO_3S$
18	OC_6H_5	*i*-Bu	K	148–150	Aq EtOH	47	$C_{17}H_{19}NO_3S$
19	OC_6H_5	*sec*-Bu	K	157–159	Me_2CO–H_2O	41	$C_{17}H_{19}NO_3S$
20	OC_6H_5	*n*-Am	L	101–102	Cyclohexane	29	$C_{18}H_{21}NO_3S$
21	OC_6H_5	*i*-Am	K	132–133	EtOH	39	$C_{18}H_{21}NO_3S$
22	OC_6H_5	$CH_2C_6H_5$	M	165–167	EtOH	65	$C_{20}H_{17}NO_3S$
23	OC_6H_5	$CH_2CCHCHSCH$	N	153	EtOH–H_2O	18[f]	$C_{18}H_{15}NO_3S_2 \cdot 0.5H_2O$
24	OC_6H_4, 4-OMe	$CH_2C_6H_5$	M	158–161	EtOH–H_2O	37	$C_{21}H_{19}NO_4S$
25	SC_6H_5	*n*-Bu	K	159–160	Aq EtOH	26	$C_{17}H_{19}NO_2S_2$
26	SC_6H_5	$CH_2C_6H_5$	M	170–172	EtOH	26	$C_{20}H_{17}NO_2S_2$
27	SC_6H_4, 2-Me	$CH_2C_6H_5$	M	196–197	Aq EtOH	60	$C_{21}H_{19}NO_2S_2$
28	SC_6H_5, 4-Me	$CH_2C_6H_5$	M	177–179	Aq EtOH	24	$C_{21}H_{19}NO_2S_2$

[a,b]See corresponding footnotes in Table I. [c]See footnote *c*, Table I, except that the compounds were dried *in vacuo* at 65–78° unless otherwise stated. [d]The compounds were analyzed for C, H, and N. Analytical results are within 0.4% of the theoretical values unless otherwise stated. [e]C: calcd, 63.77; found, 63.36. [f]Dried in air.

Scheme II



6-hr period is almost similar to that obtained with its sulfur analog **33** (Table V).

It is noteworthy that the 3-*n*-butylthiobenzoic acid derivative **47** is even more potent than the corresponding 3-*n*-butylamino-4-chloro-5-sulfamoylbenzoic acid, while the sulfonyl compounds **49–53** are completely devoid of activity after intraveneous application of 1 mg/kg (Table III).

In view of the equal high diuretic potency of some 4-substituted 3-amino-5-sulfamoylbenzoic acid and 5-sulfamoylanthranilic acid derivatives,[1,3-5] the most striking feature of the present investigation is that this does not apply to the series of their sulfur analogs. In contrast to the high potency found in the 3-alkylthio-5-sulfamoylbenzoic acid series, none of the 5-sulfamoylthiosalicylic acid

Scheme III

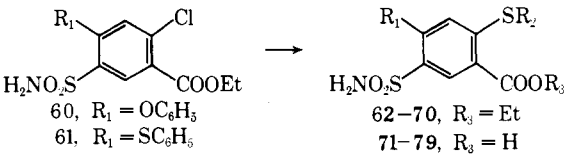
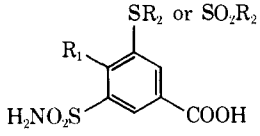


Table III. Physical Properties and Diuretic and Saluretic Activity of



No.	R_1	R_2	Method[b]	Mp, °C	Recrystn solvent[c]	Yield, %[d]	Formula[e]	Treatment,[f] mg/kg	Urinary excretion[a] ml/kg per 3 hr, H_2O	mequiv/kg per 3 hr Na^+	K^+	Cl^-
							SR_2					
29	OC_6H_5	Et	O	225–227	EtOH–H_2O	45	$C_{15}H_{15}NO_5S_2 \cdot 0.5H_2O$	0.1	6	0.5	0.2‡	0.8
30	OC_6H_5	*n*-Pr	O	209–210	Aq EtOH	28	$C_{16}H_{17}NO_5S_2 \cdot 0.5H_2O$	0.1	23	2.4	0.4	3.1
								0.1 po	22	2.4	0.5	3.4
31	OC_6H_5	$CH_2CH{=}CH_2$	O	218–219	Aq EtOH	30	$C_{16}H_{15}NO_5S_2$	0.1	12	1.1	0.2‡	1.2
32	OC_6H_5	$CH_2C{\equiv}CH$	O	197–198	EtOH–H_2O	34	$C_{16}H_{13}NO_5S_2$	0.1	14	1.3	0.3	1.9
33	OC_6H_5	*n*-Bu	O	221–222	Aq EtOH	48	$C_{17}H_{19}NO_5S_2$	0.1[g]	25.7[h] ± 3.8	2.5[h] ± 0.3	0.57[h] ± 0.08	3.5[h] ± 0.34
								0.1 po[g]	29.8[h] ± 0.8	3.3[h] ± 0.2	0.69[h] ± 0.10	3.8[h] ± 0.3
34	OC_6H_5	*i*-Bu	O	193–194	EtOH–H_2O	29	$C_{17}H_{19}NO_5S_2$	0.1	12	1.1	0.5	1.6
35	OC_6H_5	*sec*-Bu	O	192–193	Aq EtOH	27	$C_{17}H_{19}NO_5S_2$	0.1	10	0.9	0.4	1.3
36	OC_6H_5	*n*-Am	O	180–181	EtOH–H_2O	15	$C_{18}H_{21}NO_5S_2$	0.1	9	0.8	0.2‡	0.9
37	OC_6H_5	*i*-Am	O	226–227	EtOH–H_2O	33	$C_{18}H_{21}NO_5S_2$	0.1	12	1.2	0.4	1.1
								1	25	3.0	0.7	4.1
38	OC_6H_5	$CH_2C_6H_5$	P	235–236	Aq EtOH	40	$C_{20}H_{17}NO_5S_2$	0.1	7	0.8	0.3	1.3
39	OC_6H_5	$CH_2CCHCHSCH$	O	226–227	EtOH–H_2O	32	$C_{18}H_{15}NO_5S_3 \cdot H_2O$[k]	0.1	16	2.1	0.7	2.3
								0.01	9	0.9	0.2‡	1.2
40	OC_6H_5	$CH_2CCHCHCHO$	Q	216–218	Aq EtOH	19	$C_{18}H_{15}NO_6S_2$	0.1	15	1.5	0.4	2.0
41	OC_6H_5	$CH_2CH_2CCHCHNCHCH$	R	162[i]	H_2O	16[j]	$C_{20}H_{18}N_2O_5S_2 \cdot 2H_2O$[k]	0.1	17	1.6	0.3	2.0
42	OC_6H_4, 4-OMe	$CH_2C_6H_5$	O	215–216	Aq EtOH	52	$C_{21}H_{19}NO_6S_2$	0.1	7	0.4	0.2‡	0.5
43	SC_6H_5	*n*-Bu	S	192–193	Aq EtOH	71	$C_{17}H_{19}NO_4S_3 \cdot 0.5H_2O$	0.1	27	3.2	0.6	3.8
								0.01	10	1.2	0.3	1.5
								0.01 po	8	0.9	0.4	1.2
44	SC_6H_5	$CH_2C_6H_5$	T	206–208	Aq EtOH	48	$C_{20}H_{17}NO_4S_3$	0.1	14	1.7	0.3	2.0
45	SC_6H_4, 2-Me	$CH_2C_6H_5$	T	226–227	EtOH–H_2O	15	$C_{21}H_{19}NO_4S_3$	0.1	19	2.7	0.5	2.6
46	SC_6H_4, 4-Me	$CH_2C_6H_5$	T	250–251	EtOH–H_2O	14	$C_{21}H_{19}NO_4S_3 \cdot C_2H_5OH$	0.1	12	1.2	0.3	1.5
47	Cl	*n*-Bu	U	203–204	Aq EtOH	29	$C_{11}H_{14}ClNO_4S_2 \cdot 0.5H_2O$	1	25[l]	2.7[l]	0.9[l]	3.5[l]
								0.1	3[l]	0.3[l]	0.2‡[l]	0.4[l]
48	Cl	$CH_2C_6H_5$	V	246–247	Aq EtOH	71	$C_{14}H_{12}ClNO_4S_2 \cdot 0.25H_2O$[k]	1	8	0.8	0.5	1.4

						SO_2R_2						
49	Cl	*n*-Bu	W	230–231	Aq EtOH	40	$C_{11}H_{14}ClNO_6S_2$	1	As control			
50	Cl	$CH_2C_6H_5$	W	234–236[m]	EtOH–H_2O	52[j]	$C_{14}H_{12}ClNO_6S_2 \cdot H_2O$[k]	1	As control			
51	OC_6H_5	*n*-Bu	W	223–224	Aq EtOH	49[j]	$C_{17}H_{19}NO_7S_2$[n]	1	As control			
52	SC_6H_5	*n*-Bu	X	173–174	AcOH–H_2O	38	$C_{17}H_{19}NO_6S_3$	1	As control			
53	SC_6H_5	$CH_2C_6H_5$	X	230[o]	AcOH–H_2O	44	$C_{20}H_{17}NO_6S_3 \cdot 0.5H_2O$	1	As control	2.4[q]	0.44[q]	3.5[q]
3-*n*-Butylamino-4-phenoxy-5-sulfamoylbenzoic acid (bumetanide)[p]								0.1	26.0[q]	± 0.4	± 0.11	± 0.8
									± 8.3	3.3[q]	0.49[q]	4.5[q]
								0.1 po	31.0[q]	± 0.9	± 0.16	± 1.4
									± 7.6	0.92[q]	0.27[q]	1.4[q]
								0.01	10.0[q]	± 0.42	± 0.05	± 0.5
									± 4.8	0.6	0.4	1.3
3-*n*-Butylamino-4-chloro-5-sulfamoylbenzoic acid[r]								1	10	0.10[h]	0.16[h]	0.08[h]
Control									0.93[h]	±0.02	± 0.01	± 0.02
									± 0.35			

[a] The procedure is described in ref 2; when not otherwise stated single test only. Values not significantly different from controls (one-sided 95% confidence limits) are marked with a ‡. Where three or more tests were performed the average ± S.D. of the mean is given. [b] See footnote *a*, Table I. [c] See footnote *b*, Table I. [d] See footnote *c*, Table I, except that the compounds were dried *in vacuo* at 65–78° unless otherwise stated. [e] See footnote *d*, Table II. [f] When not otherwise stated iv injection in NaOH solution. [g] For dose-response in a 6-hr period, see Table V. [h] Average of three tests. [i] At about 87° change, due to evaporation of H_2O. [j] Dried in air. [k] Also analyzed for H_2O. [l] Average of two tests. [m] At about 145° change, due to evaporation of H_2O. [n] Also obtained with 1 mol of H_2O. [o] At about 128° change, due to evaporation of H_2O. [p] See ref 3. [q] Average of four tests. [r] See ref 2.

derivatives **71–79** (Table IV) showed significant diuretic activity after intravenous administration of 0.1 mg/kg. After 1 mg/kg only **76, 78,** and **79** were moderately active resulting in the 3-hr test period in the following urinary parameters per kilogram: **76,** 8 ml of urine, 0.8 mequiv of Na^+, 0.3 mequiv of K^+, and 0.8 mequiv of Cl^-; **78,** 6 ml of urine, 0.4 mequiv of Na^+, 0.4 mequiv of K^+, and 0.5 mequiv of Cl^-; **79**, 10 ml of urine, 0.4 mequiv of Na^+, 0.2 mequiv of K^+, and 0.6 mequiv of Cl^-. For control values see Table III.

Our earlier studies[1,3-5] have revealed that the dependence of the diuretic potency on structural changes tended to be less marked in the 3-amino-5-sulfamoylbenzoic acid series than in the 5-sulfamoylanthranilic acid series. The present results showed that the difference of this dependence becomes pronounced when the alkylamino function is replaced by the alkylthio side chain.

Experimental Section

Technical assistance was given by H. Dannacher, W. Schlichtkrull, J. Preisler, and Ch. Jepsen. Analyses were performed by G. Cornali and W. Egger of these laboratories. Melting points were corrected and taken in open glass capillaries using a Hershberg apparatus. For the typical compounds nmr spectra were taken by N. Rastrup Andersen on a Varian A-60A spectrometer. Spectral features were in accord with structures. For compounds not included in the tables, analyses are indicated only by symbols of the elements; analytical results were within ±0.4% of the theoretical values when not otherwise stated.

Monosodium Salt of 2-Chloro-3-nitro-5-carboxybenzenesulfinic Acid (2). A dry mixture of 4-chloro-3-chlorosulfonyl-5-nitrobenzoic acid (**1**,[2] 60 g, 0.2 mol) and Na_2SO_3 (75.5 g, 0.6 mol) was added to water (75 ml) in portions over a period of 5 hr while stirring and keeping the temperature at 10–15°. The pH of the reaction mixture was maintained at 8 by adding 2 *N* NaOH *via* an automatical end point titrator. After the NaOH uptake had ceased, the reaction mixture was filtered, and after cooling crude **2** precipitated from the filtrate by addition of concentrated HCl (60 ml). Recrystallization from H_2O yielded **2** (60%): mp 217–218°. *Anal.* ($C_7H_3ClNaO_6S \cdot 3.5H_2O$) C, H, N, H_2O.

Monosodium Salts of 2-R_1-3-Nitro-5-carboxybenzenesulfinic Acids 3–7 (Table I). Method A. To a solution of $NaHCO_3$ (21 g, 0.25 mol) in H_2O (150 ml), **2** (17.5 g, 0.05 mol) and C_6H_5OH (14.1 g, 0.15 mol) were added. The mixture was heated for 8 days at 80°. After cooling the reaction mixture was extracted three times with Et_2O (150 ml totally) and the aqueous layer acidified with concentrated HCl (20 ml) to precipitate crude **3**.

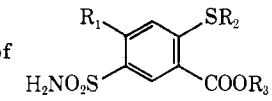
Method B. To a solution of $NaHCO_3$ (0.43 mol/0.1 mol of **2**) in H_2O (7 ml/g of **2**) **2** and the appropriate phenol (0.25 mol/0.1 mol of **2**) were added. The mixture was stirred at 80° for 4–6 days. After cooling the reaction mixture was extracted several times with Et_2O and the aqueous layer adjusted to pH 2 by addition of concentrated HCl to precipitate crude **4** which was collected after dilution with saturated NaCl due to a felt-like precipitate.

Method C. Method B was followed except that the reaction time was reduced to 17 hr and that the following mole proportions were used: **2** (3.5 g, 10 mmol), the appropriate thiophenol (10 mmol), $NaHCO_3$ (35 mmol), and H_2O (20 ml).

Method D. Method C was followed except that the aqueous layer was adjusted to pH 4.

4-R_1-5-Amino-3-mercaptobenzoic Acids 8–12 and 4-R_1-3-R_2S-5-Aminobenzoic Acids 13–28 (Table II). Method E. To a gently boiling mixture of the appropriate Na salt **3–7**, EtOH (0.5 l./0.07 mol of Na salt), and Zn powder (1.25 g-atoms/0.07 mol of Na salt), 5 *N* HCl (0.5 l./0.07 mol of Na salt) was added dropwise during 1.5–2 hr while stirring. After additional boiling and stirring for 2 hr the reaction mixture was filtered, and the EtOH was removed from the filtrate by evaporation *in vacuo*. After cooling for 24 hr, the resulting precipitate was collected, washed with H_2O, and suspended in H_2O (150–300 ml/0.07 mol of Na salt). The pH was adjusted to 2 while stirring whereafter the precipitated crude **8–12** containing amounts of the corresponding Et ester was treated with 1 *N* NaOH (5–10 ml/g) on a steam bath for 10–30 min (for **9**, 5 min due to destruction). After cooling the pH was adjusted to 2 by addition of 4 *N* HCl to precipitate crude **8–12**.

Method F. **8** (2.6 g, 10 mmol) was dissolved in H_2O (100 ml) by addition of 1 *N* NaOH (20 ml). EtI (3.12 g, 20 mmol) was added,

Table IV. Physical Properties of 

No.	R_1	R_2	Method[a]	Mp, °C	Recrystn solvent[b]	Yield, %[c]	Formula[d]
				R_3 = Et			
62	OC_6H_5	*n*-Pr	Y	155–156	EtOH	42	$C_{18}H_{21}NO_5S_2 \cdot 0.25H_2O$
63	OC_6H_5	*n*-Bu	Y	158–159	EtOH	45	$C_{19}H_{23}NO_5S_2$
64	OC_6H_5	*n*-Am	Y	158–159	EtOH	47	$C_{20}H_{25}NO_5S_2$
65	OC_6H_5	*i*-Am	Y	141–142	EtOH	29	$C_{20}H_{25}NO_5S_2$
66	OC_6H_5	$CH_2C_6H_5$	Y	187–188	*e*	59	$C_{22}H_{21}NO_5S_2$
67	OC_6H_5	$CH_2CCHCHCHO$	Y	155–156	EtOH	32	$C_{20}H_{19}NO_6S_2$
68	SC_6H_5	*n*-Bu	Y	123–125	EtOH	14	$C_{19}H_{23}NO_4S_3 \cdot 0.5H_2O$
69	SC_6H_5	$CH_2C_6H_5$	Y	172–173	EtOH	27	$C_{22}H_{21}NO_4S_3$
70	SC_6H_5	$CH_2CCHCHCHO$	Y	158–160	EtOH	26	$C_{20}H_{19}NO_5S_3$
				R_3 = H			
71	OC_6H_5	*n*-Pr	Z	215–216	Aq EtOH	72	$C_{16}H_{17}NO_5S_2 \cdot 0.25H_2O$
72	OC_6H_5	*n*-Bu	Z	194–196	Aq EtOH	70	$C_{17}H_{19}NO_5S_2 \cdot 0.5H_2O$
73	OC_6H_5	*n*-Am	Z	189–190	Aq EtOH	72	$C_{18}H_{21}NO_5S_2 \cdot 0.5H_2O$
74	OC_6H_5	*i*-Am	Z	196–197	Aq EtOH	79	$C_{18}H_{21}NO_5S_2$
75	OC_6H_5	$CH_2C_6H_5$	Z	222–223	EtOH	18	$C_{20}H_{17}NO_5S_2$
76	OC_6H_5	$CH_2CCHCHCHO$	Z	242–244 dec	Aq EtOH	36	$C_{18}H_{15}NO_6S_2$
77	SC_6H_5	*n*-Bu	Z	225–227	Aq EtOH	52	$C_{17}H_{19}NO_4S_3 \cdot 0.5H_2O$
78	SC_6H_5	$CH_2C_6H_5$	Z	225–227	EtOH	71	$C_{20}H_{17}NO_4S_3$
79	SC_6H_5	$CH_2CCHCHCHO$	Z	245–247 dec	Aq EtOH	53	$C_{18}H_{15}NO_5S_3$

[a]–[c]See corresponding footnotes in Table I. [d]See corresponding footnote in Table II. [e]A mixture of EtOH (ten parts) and methyl cellosolve (one part) was used.

and the mixture was stirred for 2 hr at room temperature while excess of EtI was allowed to evaporate. After addition of EtOH (50 ml), the pH was adjusted to 2.5 by addition of 1 *N* HCl to precipitate crude **13**.

Method G. To a solution of **8** (2.6 g, 10 mmol) in 1 *N* NaOH (20.5 ml) *n*-PrI (2.55 g, 15 mmol) was added. The reaction mixture was stirred in a closed reaction vessel for 30 hr at room temperature. After filtration and extraction of the filtrate with Et_2O, the aqueous layer was adjusted to pH 2.5 by addition of 1 *N* HCl to precipitate crude **14**.

Method H. A solution of **8** (1.3 g, 5 mmol) in 1 *N* $NaHCO_3$ (60 ml) was cooled to 5°. After addition of $CH_2{=}CHCH_2Br$ (0.51 g, 4.2 mmol) the reaction mixture was stirred for 10 min while cooled by ice. Addition of 4 *N* HCl until pH 2.5 precipitated crude **15**.

Method I. To a solution of **8** (1.3 g, 5 mmol) in 1 *N* $NaHCO_3$ (60 ml) $CH{\equiv}CCH_2Br$ (0.6 g, 5 mmol) was added and the reaction mixture stirred at 60° for 1 hr. After cooling, neglecting precipitated amorphous Na salt of **16**, the reaction mixture was adjusted to pH 2.5 by addition of 4 *N* HCl to precipitate crude **16**. It was dissolved in hot saturated $NaHCO_3$ (12 ml), followed by addition of saturated NaCl (12 ml). Cooling precipitated the Na salt of **16**. It was redissolved in hot H_2O (40 ml) and crude **16** liberated by addition of 4 *N* HCl until pH 2.5.

Method J. To a solution of **8** (26.1 g, 0.1 mol) in 1 *N* $NaHCO_3$ (500 ml) *n*-BuI (22 g, 0.12 mol) was added and the mixture stirred in a N_2 atmosphere at 50° for 5 hr. After cooling the pH was adjusted to 2.5 by addition of 4 *N* HCl to precipitate crude **17**.

Method K. Method J was followed using the appropriate alkyl iodide (12 mmol/10 mmol of **8**) and 1 *N* $NaHCO_3$ (100 ml/10 mmol of **8**). For **18** additional *i*-BuI (5 mmol) was added during the reaction.

Method L. A mixture of **8** (0.65 g, 2.5 mmol), 1 *N* NaOH (10 ml), and *n*-AmBr (0.75 g, 5 mmol) was stirred at 50–55° for 20 hr. After cooling the mixture was extracted with Et_2O, and the aqueous layer was adjusted to pH 2.5 by addition of 4 *N* HCl to precipitate crude **20**.

Method M. To a solution of the appropriate 4-R_1-5-amino-3-mercaptobenzoic acid (2–10 mmol) in 1 *N* $NaHCO_3$ (100 ml/10 mmol of mercaptobenzoic acid) $C_6H_5CH_2Br$ (10–12 mmol/10 mmol of mercaptobenzoic acid) was added, and the reaction mixture was stirred at room temperature for about 17 hr. Neglecting casual precipitation of the Na salt the pH was adjusted to 2.5 to precipitate the crude material. For **22**, the Na salt was collected and worked up adapting method I.

Method N. To a solution of **8** (2.6 g, 10 mmol) in 0.5 *N* NaOH (100 ml) a solution of 3-thenyl bromide[7] (about 3.6 g, 20 mmol) in benzene (20 ml) was added, and the mixture was stirred at room temperature for 1 hr. After removing the benzene, 1 *N* HCl (50 ml) was added to precipitate crude **23**.

4-R_1-3-R_2S-5-Sulfamoylbenzoic Acids 29–53 (Table III). Method O. A solution of the appropriate 4-R_1-3-R_2S-5-aminobenzoic acid (4–10 mmol) in 1 *N* NaOH (1 ml/1 mmol of aminobenzoic acid) and $NaNO_2$ (4–10 mmol) was, neglecting casual precipitation of the appropriate Na salt, at 2–5° added dropwise to a stirred mixture of equal parts of AcOH and concentrated HCl (about 2.5 ml/1 mmol of aminobenzoic acid). The resulting diazonium mixture was poured into AcOH (2.5 ml/1 mmol of aminobenzoic acid) saturated with SO_2 and containing $CuCl_2 \cdot 2H_2O$ (about 0.025 g/1 mmol of aminobenzoic acid). The reaction mixture was allowed to reach room temperature while stirring for several hours. After cooling, the precipitated sulfochloride was dried *in vacuo* at room temperature and poured into concentrated aqueous NH_3 (about 20 ml/g of sulfochloride) while stirring. After additional stirring for 1 hr, the reaction mixture was heated on a steam bath for 1 hr allowing most of the excess of NH_3 to evaporate. Cooling precipitated NH_4 salt. Redissolving in hot H_2O and acidification with an excess of 4 *N* HCl precipitated the crude sulfamoyl compound. For **39** the equivalent amount of KNO_2 and KOH was used in the diazotation step. For **31**, **32**, **34–36**, and **39** the reaction mixture after the amidation process was acidified without isolation of an NH_4 salt.

Method P. Method O was followed except that the equivalent amount of KNO_2 and KOH was used and that the reaction mixture obtained after the amidation process was acidified directly. The first purification was performed *via* the Na salt which was precipitated on cooling a solution of the crude product in an excess of hot 1 *N* $NaHCO_3$.

Method Q. A mixture of **56** (1.6 g, 5 mmol), trimethyl(2-furylmethyl)ammonium iodide (2 g, 7.5 mmol), K_2CO_3 (0.5 g, 3.75 mmol), and diglyme (15 ml) was stirred at 110° for 4 hr. After cooling 1 *N* KOH (25 ml) and H_2O were added, and the resulting solution was acidified with AcOH. Extraction with AcOEt, followed by evaporation *in vacuo*, and trituration with aqueous Me_2CO yielded crude **40**.

Method R. 4-Vinylpyridine (0.21 g, 2 mmol) was added to a solution of **56** (0.33 g, 1 mmol) in 1 *N* $NaHCO_3$ (10 ml) and the mixture was kept at 80° for 3 hr. After cooling crude **41** was precipitated by addition of aqueous AcOH.

Method S. A solution of **47** (0.78 g, 2.35 mmol) in saturated

Table V. Diuretic and Saluretic Activity of Compound **33**

Treatment, mg/kg	Urinary excretion[a]							
	iv[b]				po[c]			
	ml/kg per 6 hr, H_2O	mequiv/kg per 6 hr			ml/kg per 6 hr, H_2O	mequiv/kg per 6 hr		
		Na^+	K^+	Cl^-		Na^+	K^+	Cl^-
Control	1.73 ± 0.46	0.27 ± 0.09	0.24 ± 0.03	0.17 ± 0.09				
0.01	9.86 ± 0.49	0.81 ± 0.08	0.30 ± 0.01‡	0.98 ± 0.11	6.60 ± 0.58	0.78 ± 0.06	0.25 ± 0.03‡	1.00 ± 0.08
0.1	28.9 ± 5.0	2.82 ± 0.42	0.75 ± 0.10	3.86 ± 0.56	37.0 ± 1.6	3.91 ± 0.20	1.03 ± 0.17	4.71 ± 0.40
0.25	38.6 ± 2.7	4.20 ± 0.39	0.96 ± 0.06	5.40 ± 0.25	45.7 ± 4.7	4.66 ± 0.57	1.37 ± 0.06	6.37 ± 0.66
0.5	59.3 ± 5.0	5.25 ± 0.48	1.37 ± 0.13	7.16 ± 0.67	42.0 ± 5.0	4.31 ± 0.52	1.20 ± 0.19	5.76 ± 0.78
1.0	58.1 ± 4.4	5.59 ± 0.65	1.46 ± 0.05	7.29 ± 0.76	55.4 ± 9.9	5.85 ± 1.04	1.52 ± 0.13	7.48 ± 1.43

[a] The procedure is described in ref 2. The average of three tests ± S.D. of mean is given. Values not significantly different from controls (one-sided 95% confidence limits) are marked with a ‡. [b] In NaOH solution. [c] In gelatin capsules.

ethanolic HCl (35 ml) was kept for 18 hr at room temperature. The precipitated ethyl ester of **47** was washed with EtOH and petroleum ether and refluxed for 6 hr in EtOH (25 ml) containing C_6H_5SNa (5 mmol). After evaporation *in vacuo* the residue was saponified by refluxing in a mixture of EtOH (10 ml) and 1 *N* NaOH (20 ml) for 15 min. After cooling and extraction with Et_2O, the aqueous layer was acidified by addition of 4 *N* HCl to precipitate crude **43**.

Method T. A mixture of the appropriate 4-R_1-3-R_2S-5-aminobenzoic acid (2 mmol), AcOH (10 ml), and concentrated HCl (10 ml) was cooled to 0–5°. A solution of $NaNO_2$ (0.14 g, 2 mmol) in H_2O (1 ml) was added dropwise while stirring and keeping the temperature. The resulting diazonium mixture was poured into AcOH (25 ml) saturated with SO_2 and containing $CuCl_2 \cdot 2H_2O$ (0.25 g). The reaction mixture was worked up and the isolated crude sulfochloride allowed to react with concentrated aqueous NH_3 as given under method O, **46**, without isolation of an NH_4 salt.

Method U. Method G was adapted using **59** and *n*-BuI precipitating the Na salt of **47**. Redissolving in hot H_2O and acidification with 4 *N* HCl precipitated crude **47**.

Method V. A mixture of **59** (1.34 g, 5 mmol), $C_6H_5CH_2Br$ (1.4 g, 8 mmol), and saturated $NaHCO_3$ (20 ml) was stirred at room temperature for 3 hr to precipitate the Na salt of **48**. Redissolving in hot aqueous EtOH and addition of 4 *N* HCl precipitated crude **48**.

Method W. A mixture of the appropriate 3-R_2S-benzoic acid (2 mmol), AcOH (7.5 ml), and H_2O_2 (30% aqueous solution, 2 ml) was stirred for 60–70 hr at room temperature to precipitate the crude reaction product.

Method X. To a solution of the appropriate 4-chlorobenzoic acid (3 mmol) in 1 *N* $NaHCO_3$ (30 ml) C_6H_5SH (10 mmol for **52**, 6 mmol for **53**) was added and the mixture stirred at 80° for 2–3 hr. After cooling the reaction mixture was made alkaline (NaOH) and extracted twice with Et_2O (50 ml). For **52** the aqueous layer was adjusted to pH 7 by addition of 4 *N* HCl to precipitate the Na salt of **52**, which was worked up according to method U. For **53** the aqueous layer was acidified to precipitate crude **53**.

3,3′-Diamino-5,5′-dicarboxy-2,2′-diphenoxydiphenyl Disulfide (54). To a stirred solution of **9** (15.7 g, 0.06 mol) in a mixture of 1 *N* NaOH (150 ml) and EtOH (150 ml) a solution of iodine (7.6 g, 0.06 mol) in EtOH (200 ml) was added at room temperature. After 30 min H_2O (600 ml) and 4 *N* HCl (100 ml) were added to precipitate crude **54**. Recrystallization from EtOH–H_2O while treating with decolorizing C and drying *in vacuo* at 80° yielded **54** (70%), mp 280–282° dec. *Anal.* ($C_{26}H_{20}N_2O_6S_2$) C, H, N.

5,5′-Dicarboxy-3,3′-dichlorosulfonyl-2,2′-diphenoxydiphenyl Disulfide. A solution of **54** (5.2 g, 10 mmol) in 1 *N* NaOH (23 ml) containing $NaNO_2$ (1.54 g, 22 mmol) was at 0–5° added dropwise to a stirred mixture of AcOH (25 ml) and concentrated HCl (25 ml). After additional stirring at 5° for 30 min the resulting diazonium mixture was at room temperature poured into stirred AcOH (65 ml), saturated with SO_2 and containing $CuCl_2 \cdot 2H_2O$ (0.65 g) previously dissolved in H_2O (2 ml). The reaction mixture was stirred for several hours to precipitate crude sulfochloride (92%), mp 220° dec. A sample was recrystallized several times from AcOH–H_2O and once from $CHCl_3$–petroleum ether, mp 234°. *Anal.* ($C_{26}H_{16}Cl_2S_4O_{10}$) C, H, Cl; S: calcd, 18.65; found, 17.90.

5,5′-Dicarboxy-2,2′-diphenoxy-3,3′-disulfamoyldiphenyl Disulfide (55). 5,5′-Dicarboxy-3,3′-dichlorosulfonyl-2,2′-diphenoxydiphenyl disulfide (3.45 g, 5 mmol) was added in portions to liquid NH_3 while stirring. Excess NH_3 was allowed to evaporate at room temperature. The residue was washed with Et_2O (50 ml) and dissolved in H_2O. Addition of 4 *N* HCl (5 ml) precipitated crude **55**. Recrystallization from aqueous EtOH, while treating with decolorizing C, and drying in air yielded **55** (58%), mp >300°. *Anal.* ($C_{26}H_{20}N_2O_{10}S_4 \cdot 1.75H_2O$) C, H, N.

3-Mercapto-4-phenoxy-5-sulfamoylbenzoic Acid (56). To a stirred solution of **55** (1.3 g, 2 mmol) in saturated $NaHCO_3$ (12.4 ml), $Na_2S_2O_4$ (2.5 g, 14 mmol) was added in portions under N_2. After 3 hr the reaction mixture was acidified by addition of 4 *N* HCl to precipitate crude **56** (92%, dried *in vacuo* at 80°), **56** was alkylated without further purification, due to partial disulfide formation during recrystallization.

5,5′-Dicarboxy-2,2′-dichloro-3,3′-disulfamoyldiphenyl Disulfide (58). **57**[2] (25 g, 0.1 mol) was treated with concentrated HCl (25 ml), followed by H_2O (200 ml), and at 5° diazotized by addition of $NaNO_2$ (6.9 g, 0.1 mol) in H_2O (20 ml). The resulting diazonium mixture was added dropwise to a solution of Na_2S_2 (prepared from 27 g of $Na_2S \cdot 9H_2O$, 3.5 g of S, 46 g of 30% NaOH, and

50 ml of H_2O) while stirring and keeping the temperature between 1 and 3°. After stirring for an additional 16 hr and allowing the mixture to reach room temperature, the pH was adjusted to 7.5 by addition of 4 *N* HCl. After filtration the filtrate was acidified with 4 *N* HCl. The resulting amorphous precipitate was crystallized by trituration with MeOH (200 ml), recrystallized from aqueous EtOH, washed thoroughly with MeOH (200 ml), and dried *in vacuo* at 80° to yield **58** (24%), mp 221°. *Anal.* ($C_{14}H_{10}Cl_2N_2O_8S_4$) C, H, N.

4-Chloro-3-mercapto-5-sulfamoylbenzoic Acid (59). To a stirred solution of **58** (1.6 g, 3 mmol) in 1 *N* $NaHCO_3$ (50 ml), $Na_2S_2O_4$ (6 g, 34 mmol) was added in portions followed by heating on a steam bath for 30 min. Cooling, acidification with 4 *N* HCl, and recrystallization of the resulting precipitate from Me_2CO–petroleum ether yielded crude **59** (68%), mp 268–269°, which was used without further purification. For analysis a sample was recrystallized several times from EtOH–H_2O and MeOH–H_2O, mp 277.5–278°. *Anal.* ($C_7H_6ClNO_4S_2$) C, H, N.

Ethyl **2-Chloro-4-phenoxy-5-sulfamoylbenzoate (60).** 2-Chloro-4-phenoxy-5-sulfamoylbenzoic acid[4] was esterified in EtOH using concentrated H_2SO_4 as catalyst. Concentration *in vacuo* and addition of H_2O precipitated crude **60**. It was recrystallized from aqueous EtOH and dried *in vacuo* to yield **60** (72%), mp 143–145°. *Anal.* ($C_{15}H_{14}ClNO_4S$) C, H, Cl, N, S.

Ethyl **2-Chloro-4-phenylthio-5-sulfamoylbenzoate (61).** 2-Chloro-4-phenylthio-5-sulfamoylbenzoic acid[4] was esterified as described for **60**. Crude **61** precipitated on concentration. It was recrystallized from EtOH to yield **61** (76%), mp 162–164°. *Anal.* ($C_{15}H_{14}ClNO_3S_2$) C, H, Cl, N, S.

Ethyl **R_2S-4-R_1-5-Sulfamoylthiosalicylates 62–70 and R_2S-4-R_1-5-Sulfamoylthiosalicylic Acids 71–79 (Table IV). Method Y.** To a solution of NaOEt (prepared from 11 mmol of Na) in dry EtOH (10–18 ml), **60** or **61** (5 mmol) was added followed by the appropriate R_2SH (5.5 mmol), and the mixture was refluxed for 4–6 hr. After addition of concentrated HCl (1.0 ml) or AcOH (1.0 ml) and cooling, the crude reaction product crystallized, eventually after dilution with H_2O. The material was washed with H_2O and dried in air, prior to recrystallization.

Method Z. The appropriate Et ester **62–70** was saponified with an excess of 2 *N* NaOH by heating on a steam bath for 15 min. After cooling, the crude reaction product was precipitated by acidification with an excess of 4 *N* HCl or 4 *N* AcOH.

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Puromycin Analogs.[1] Studies on Ribosomal Binding with Diastereomeric Carbocyclic Puromycin Analogs†

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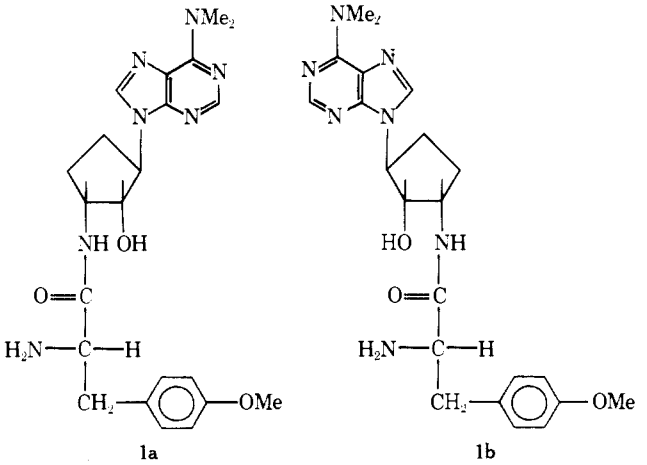
A direct and convenient route to the antimicrobial carbocyclic puromycin analog, 6-dimethylamino-9-[(*R*)-[(2*R*)-hydroxy-(3*R*)-(*p*-methoxyphenyl-L-alanylamino)]cyclopentyl]purine (**1a**), is described. Epoxidation of 3-acetamidocyclopentene (**3**) gave exclusively *cis*-3-acetamido-1,2-epoxycyclopentane (**4**). Opening of the epoxide with NaN_3, followed by reduction of the resulting azido alcohol **5**, gave a high yield of 2α-acetamido-5β-aminocyclopentan-1α-ol (**6**). This amine was easily resolved *via* tartrate formation. Introduction of the purine moiety by standard methods gave the enantiomeric carbocyclic aminonucleosides (−)- and (+)-2α-acetamido-5β-(6-dimethylamino-9-purinyl)-cyclopentan-1α-ol (**10a** and **10b**). Resolution at an early point allows for the conversion of **10a** and **10b** to a wide variety of diastereomeric aminoacyl derivatives. Studies on protein synthesis inhibition with diastereomeric carbocyclic puromycin analogs indicate that two distinct types of protein synthesis inhibitors may have been developed—series **a** which are peptidyl transferase substrates, and series **b** which are peptidyl transferase inhibitors.

The carbocyclic puromycin analog **1a** exhibits potent antimicrobial activity[2] and is effective against three tumor lines tested in tissue culture[3] while the diastereomer **1b** was only slightly active. *In vitro* testing demonstrated that **1a** inhibits the formation of polyphenylalanine in the *Escherichia coli* cell-free system[3] and that it is an effective competitive inhibitor of puromycin for peptidylpuromycin synthesis.[4] The inhibition is stereospecific with the diastereomer **1b** being much less active than **1a**. The carbocyclic puromycin analog has only slightly less affinity for ribosomes than does puromycin itself.[4] In addition, **1a**, but not **1b**, was shown to accept acetylphenylalanine from acetylphenylalanyl-tRNA.[4] These results firmly establish that **1a** has a mechanism of action identical with that of puromycin and that structural manipulation to obtain various active analogs may be extremely useful in elucidating various aspects of protein biosynthesis.



1a **1b**

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