Potential Bis-Alkylating Agents for Cancer Chemotherapy. Approaches to the Synthesis of 2-Sulfonyl-1,4-bis(methanesulfonoxy)butanes[1]

James M. Wilbur, Jr.

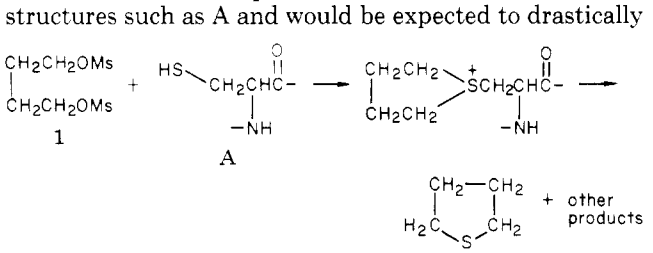
Department of Chemistry, Southwest Missouri State University, Springfield, Missouri 65802. *Received January 5, 1978*

Three general methods for the synthesis of derivatives of the bis-alkylating agent, myleran [1,4-bis(methanesulfonoxy)butane (1)], are presented. These derivatives contain a lipophilic group attached to the 2 position of 1,4-bis(methanesulfonoxy)butane by means of a sulfonyl function. As examples of the scope of the methods, the synthesis of seven such derivatives (**12a–g**) is presented. All seven were inactive against L1210 and P388 leukemia in the mouse.

Myleran (**1**) is a cytotoxic bis-alkylating agent used clinically for treatment of chronic granulocytic leukemia.[2] A study by Hudson et al.[3] using bis(methanesulfonate) esters of the type $MsO(CH_2)_nOMs$ indicated a parabolic relationship between the ether–water partition coefficients and relative activity as measured by the growth-inhibiting action on the Walker carcinosarcoma 256 (W-256) and by the neutrophil depression. Peak activity was shown by **1** ($n = 4$), and even though the water solubility of the derivative where $n = 6$ was twice that of **1**, the neutrophil depressing action was about six times less than that of **1**. Hansch et al.,[4] in a study of the structure–activity relationships of nitrosoureas and imidazolecarboxamides in the treatment of L1210 leukemia in mice, found the activity of each type of drug to be parabolically related to lipophilic character (defined by log P where P is the octanol–water partition coefficient). They assign a log P value of −0.47 for **1** in this system and point out that this coincides with the optimum log P of −0.6 found for the nitrosoureas, and, therefore, a similarity in the mechanism of action is suggested. The inactivity of **1** against the L1210 mouse leukemia has been ascribed by Elson[5] to be due to its very selective action on granulocytic leukemia as indicated by the selective depression of neutrophils and platelets more than lymphocytes.

In contrast to the above studies is that of Sandberg et al.[6] on the relationship of the structure of bis(methanesulfonate) esters to antitumor activity which shows the more lipophilic member of this series and the one with the lowest neutrophil depressing action, 1,9-bis(methanesulfonoxy)nonane ($n = 9$), to be active not only against L1210 but also against W-256. A study of the structure–activity relationships in antitumor aniline mustards indicates that solid tumors (W-256) may respond better to more lipophilic drugs than leukemias.[7] These conflicting results seem to indicate that, at least for the bis(methanesulfonate) esters, there is no absolute relationship between structure and the degree of uniformity of response in the various tumor screens.

The selectivity of **1** toward the –SH groups of cysteine, glutathione, and other sulfhydryl-containing compounds both in vitro[8,9] and in vivo[10] is well documented. It has been suggested[8] that the unique "sulfur-stripping" ability of **1** may be responsible for the activity of such bis-alkylating agents in cancer chemotherapy, since this results in the irreversible depletion or removal of sulfur from structures such as A and would be expected to drastically alter the structure and function of biologically important compounds (enzymes) containing this structural feature.



Since the unique action of **1** is related to its ability to form a cyclic five-membered sulfonium ion intermediate,[8] it appeared that derivatives having four methylene groups not contained in a cyclic structure would be required if this mechanism of action were to remain a viable possibility. Also, the terminal ester groups should be bonded to primary-type carbons since more hindered secondary or tertiary positions would retard attack by nucleophiles. The presence of a lipophilic group at the second carbon atom might enhance the passage of the alkylating agent through more lipophilic barriers and result in increased penetration of other types of tumors than leukemia.

The reported[6] derivatives with four carbon atoms separating the ester group lack these essential features and show lower activity in the various antitumor screens than the more lipophilic 1,9-bis(methanesulfonoxy)nonane. Therefore, it was of interest to investigate approaches to the synthesis of such a series of derivatives (**12a–g**, Table I). The attachment of the lipophilic group to the 2 position by means of an electron-withdrawing sulfonyl group caused some concern since it could conceivably alter the unique sulfur-stripping mechanism. In order to examine this possibility, one such derivative, **12a**, was treated with L-cysteine ethyl ester under the same conditions previously used with **1**.[8] As in the previous case, a complex mixture resulted from which *dl*-3-(*n*-butylsulfonyl)-tetrahydrothiophene was obtained (37.6% yield) as the only characterizable product.[11] Recrystallization from ether afforded a sample (mp 51–52 °C) with the correct analysis (C, H, and S). This result is in good accord with the previous study in which the sulfur-elimination product, tetrahydrothiophene, was obtained in 12% yield.[8a] This seems to lessen serious concern about the ability of the sulfonyl group to drastically alter this unique mechanism of action. Admittedly, there is no assurance that the same results would be obtained with the other analogues or under in vivo conditions.

Chemistry. The three approaches (A–C) to the synthesis of **12a–g** are outlined in Scheme I. The common feature of all routes is the preparation of a series of intermediate 2-(substituted thio)-1,4-butanediols **10a–g**.

Method A involves the free-radical addition of *n*-alkylthiols **2a,b** to *cis*-1,4-diacetoxy-2-butene. The products, 2-(alkylthio)-1,4-diacetoxybutanes **4a,b**, are formed as a mixture of optical isomers and are converted to **10a,b** by alkaline hydrolysis. The simplicity of the method and good product yields are attractive features of method A, which is the preferred route when the *n*-alkylthiols are available.

Method B is suitable for the more reactive thiols like thiophenol and benzyl mercaptan which undergo base-catalyzed addition to the readily available diethyl maleate. The thio diols **10c,d** are then formed by lithium aluminum hydride ($LiAlH_4$) reduction of the diesters **7c,d**.

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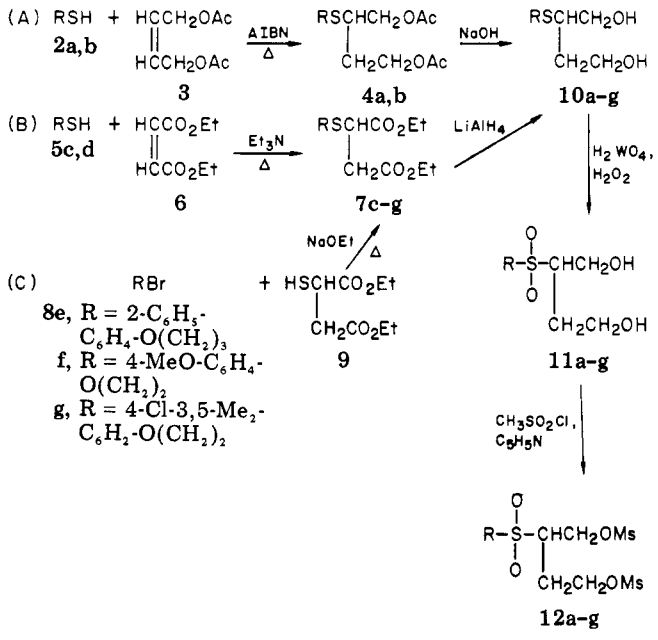
Table I. 2-(Substituted sulfonyl)-1,4-bis(methanesulfonoxy)butanes and Their Precursors

compd	R	% yield (reaction solvent)	mp or bp (mm), °C	recrystn solvent	formula	analyses[a]
4a	C_4H_9	81	138 (1.5)		$C_{12}H_{22}SO_4$	H, S; C[b]
4b	$C_{11}H_{23}$	66	182-183 (0.55)		$C_{19}H_{36}SO_4$	C, H, S
7c	C_6H_5	90	155-157 (1.1)		$C_{14}H_{18}SO_4$	c
7d	$C_6H_5CH_2$	81	148-149 (0.25)		$C_{15}H_{20}SO_4$	c
7e	2-C_6H_5-C_6H_4-$O(CH_2)_3$	92	syrup		d	
7f	4-MeO-C_6H_4-$O(CH_2)_2$	90	44-45	EtOH	$C_{17}H_{24}SO_5$	C, H, S
7g	4-Cl-3,5-Me_2-C_6H_2-$O(CH_2)_2$	89	syrup		d	
10a	C_4H_9	93.5	129-130 (0.55)		$C_8H_{18}SO_2$	C, H, S
10b	$C_{11}H_{23}$	72	53-54	C_6H_{12}	$C_{15}H_{32}SO_2$	C, H, S
10c	C_6H_5	85	167 (0.75)		$C_{10}H_{14}SO_2$	C, H, S
10d	$C_6H_5CH_2$	84	164-165 (0.1)		$C_{11}H_{16}SO_2$	C, H, S
10e	2-C_6H_5-C_6H_4-$O(CH_2)_3$	86	44-47	C_6H_6-ligroine	$C_{19}H_{24}SO_2$	C, H, S
10f	4-MeO-C_6H_4-$O(CH_2)_2$	75	syrup		d	
10g	4-Cl-3,5-Me_2-C_6H_2-$O(CH_2)_2$	90	65-67	C_6H_6-ligroine	$C_{14}H_{21}ClSO_3$	C, H, Cl, S
11a	C_4H_9	75 (acetone)	syrup		d	
11b	$C_{11}H_{23}$	94 (acetone)	syrup		d	
11c	C_6H_5	98 (EtOH)	syrup		d	
11d	$C_6H_5CH_2$	77 (acetone)	80.5-82.5	CH_2Cl_2-pet. ether	$C_{11}H_{16}SO_4$	C, H, S
11e	2-C_6H_5-C_6H_4-$O(CH_2)_3$	87 (dioxane)	84.5-85.5	C_6H_6	$C_{19}H_{24}SO_5$	C, H, S
11f	4-MeO-C_6H_4-$O(CH_2)_2$	70 (acetone)	syrup		d	
11g	4-Cl-3,5-Me_2-C_6H_2-$O(CH_2)_2$	83 (dioxane)	110-111.5	C_6H_6	$C_{14}H_{21}ClSO_5$	C, H, Cl, S
12a	C_4H_9	46	61-63	EtOH	$C_{10}H_{22}S_3O_8$	C, H, S
12b	$C_{11}H_{23}$	24	73-74.5	MeOH	$C_{17}H_{36}S_3O_8$	C, H, S
12c	C_6H_5	68	96-97	EtOH	$C_{12}H_{18}S_3O_8$	C, H, S
12d	$C_6H_5CH_2$	66	85.5-87	EtOH	$C_{13}H_{20}S_3O_8$	C, H, S
12e	2-C_6H_5-C_6H_4-$O(CH_2)_3$	69	82-83	$CHCl_3$-Et_2O	$C_{21}H_{28}S_3O_9$	C, H, S
12f	4-MeO-C_6H_4-$O(CH_2)_2$	86	81-82	$CHCl_3$-EtOH	$C_{15}H_{24}S_3O_{10}$	C, H, S
12g	4-Cl-3,5-Me_2-C_6H_2-$O(CH_2)_2$	43	111.5-112.5	$CHCl_3$-Et_2O	$C_{16}H_{25}ClS_3O_9$	C, H, Cl, S

[a] Elemental analyses of indicated elements are within ±0.4% of the theoretical values unless otherwise noted. [b] C: calcd, 54.93; found, 54.41. [c] Structure confirmed by conversion to diacid; see the Experimental Section. [d] Not analyzed.

Scheme I



Method C uses an initial Williamson ether synthesis to prepare (ω-bromoalkoxy)benzenes 8 which, upon subsequent alkylation of ethyl mercaptosuccinate, afford the diesters **7e–g**. Reduction of these with $LiAlH_4$ gives the thio diols **10e–g**. Method C has the greatest potential for introducing a variety of substituents in the 2 position.

The sulfonyl diols **11a–g** were obtained by the tungstic acid catalyzed hydrogen peroxide oxidation[12] of the thio diols. The conventional oxidation using hydrogen peroxide in acetic acid or acetone solution gave poor results. Conversion to the dimesylates by conventional methods affords the crystalline 2-sulfonyl-1,4-bis(methanesulfonoxy)butanes **12a–g** as a mixture of optical isomers.

Biological Results. Compounds **12a–g** were subjected to preliminary in vivo antitumor assay against L1210 and P388 leukemia in the mouse by the Drug Evaluation Branch of the National Cancer Institute but showed no activity at the highest tolerated dose.

Experimental Section

Boiling points are uncorrected. Fractional distillations were performed with a 190 × 12 mm helix-packed column. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. 37921, and were within ±0.4% of the calculated values unless otherwise noted. A Perkin-Elmer 337 grating spectrophotometer was used to obtain IR spectra. NMR spectra were obtained with a Varian EM 360 spectrometer.

Method A. General Preparation of 2-(Alkylthio)-1,4-diacetoxybutanes 4a,b. The following example illustrates the procedure.

***dl*-2-(*n*-Butylthio)-1,4-diacetoxybutane (4a).** A mixture of 19.8 g (0.22 mol) of butanethiol, 34.4 g (0.2 mol) of *cis*-1,4-diacetoxy-2-butene[13] [bp 78–79 °C (1.4 mm); n^{25}_D 1.4410], and 401 mg of azobis(isobutyronitrile) (AIBN) was heated with stirring under nitrogen for 24 h at 85–95 °C. Fractionation of the reaction mixture afforded 42.6 g (81.5%) of **4a**: bp 130–132 °C (1.0 mm); n^{25}_D 1.4630; IR (neat) 2960, 2940, 2870, 1740 (C=O), 1240, and 1040 cm^{-1}. A sample, bp 138 °C (1.5 mm), n^{25}_D 1.4630, was submitted for analysis. Anal. Calcd for $C_{12}H_{22}SO_4$: C, 54.93; H, 8.45; S, 12.22. Found: C, 54.41; H, 8.32; S, 12.51.

Method B. General Preparation of Diethyl 2-(Substituted thio)succinates 7c,d. The following example is illustrative.

Diethyl *dl*-2-(Benzylthio)succinate (7d). A mixture of freshly distilled benzyl mercaptan (24.8 g, 0.2 mol) and diethyl maleate (34.4 g, 0.2 mol) was added over 30 min to a stirred solution of 1 mL of triethylamine and 100 mL of dry benzene. The mixture was heated at reflux for 5 h and an additional 1 mL of triethylamine was added after 2.5 h. After cooling, the reaction mixture was washed with 5% HCl, 5% $NaHCO_3$, and water. After drying ($MgSO_4$), the benzene was removed in vacuo and the

residual oil was fractionated to afford 47.9 g (81%) of **7d**: bp 148–149 °C (0.25 mm) [lit.[14] bp 136–140 °C (0.35 mm)]; n^{25}_D 1.5150. The structure of **7d** was confirmed by alkaline hydrolysis to the diacid. This afforded, after recrystallization from water, a sample (mp 186–187.5 °C) which gave a mixture melting point (186–188 °C) with an authentic sample of 2-(benzylthio)succinic acid (mp 187–188 °C)[15], prepared by reaction of benzyl chloride with thiosuccinic acid in NaOH solution.

Compound **7c** was obtained similarly and its structure confirmed by conversion to the diacid (mp 109–111 °C) which gave a mixture melting point (109–111 °C) with an authentic[16] sample (mp 110–111 °C).

***dl*-2-(*n*-Butylthio)-1,4-butanediol (10a).** This procedure was also used for the preparation of **10b**. A mixture of 70.7 g (0.269 mol) of **4a** in a solution of 140 mL of water, 22 g (0.55 mol) of NaOH, and enough MeOH to give a homogeneous solution was stirred at reflux temperature for 3 h. The product was extracted into Et_2O. The combined Et_2O extracts were dried ($MgSO_4$) and the Et_2O was removed in vacuo. The residual oil, 47.4 g (99%), upon fractional distillation afforded 44.0 g (93.5%) of **10a**: bp 135–137 °C (0.90 mm); n^{25}_D 1.4950. A further distillation afforded an analytical sample: bp 129–130 °C (0.55 mm); n^{25}_D 1.4949. Anal. ($C_8H_{18}SO_2$) C, H, S.

Method C. (ω-Bromoalkoxy)benzenes 8e–g. The following intermediates were prepared from the appropriate potassium phenoxide and dibromoalkane according to the method of Leonard and Wildman[17] and were purified by distillation prior to use. **2-(3-Bromopropyloxy)biphenyl (8e)**: bp 140–141 °C (0.05 mm); n^{25}_D 1.6030; NMR 1.98 (m, 2 H), 3.28 (t, 2 H), 3.9 ppm (t, 2 H). Anal. ($C_{15}H_{15}BrO$) C, H. **1-Bromo-2-(4-methoxyphenoxy)-ethane (8f)**: bp 107–110 °C (0.15 mm); mp 50–51 °C (EtOH) [lit.[17] mp 51.5–52.5 °C (EtOH)]. **1-Bromo-2-(4-chloro-3,5-dimethylphenoxy)ethane (8g)**: mp 52–54 °C; bp 110–115 °C (0.15 mm) [lit.[18] bp 108–112 °C (0.04 mm)]. The following example illustrates the general procedure used to alkylate ethyl mercaptosuccinate.

Diethyl *dl*-2-[2-[(4-methoxyphenoxy)ethyl]thio]succinate (7f). Ethyl mercaptosuccinate[19] (20.6 g, 0.1 mol) was added to a solution of NaOEt, prepared from 2.3 g of Na and 100 mL of absolute EtOH. A hot solution of the bromide **8f** (23.1 g, 0.1 mol) in 100 mL of absolute EtOH was then added and the mixture was heated at reflux for 5 h. The hot solution was filtered to remove NaBr and the filtrate was concentrated in vacuo on a steam bath to about 100 mL. The product separated on cooling and was collected, washed with water, and air-dried to afford 32.1 g (90%) of **7f**, mp 43–45 °C. Two additional recrystallizations from EtOH afforded an analytical sample, mp 44–45 °C. Anal. ($C_{17}H_{24}SO_5$) C, H, S.

Compounds **7e** and **7g** were isolated by removal of most of the reaction solvent, followed by addition of water and extraction with CH_2Cl_2. After drying ($MgSO_4$) and distillation, the products (**7e,g**) were obtained as syrups. For **7g** the reaction solvent was EtOH–DMF (1:1).

***dl*-2-(Benzylthio)-1,4-butanediol (10d).** The other alkylthio diols (**10c,e–g**) were prepared in a similar manner by $LiAlH_4$ reductions of the corresponding esters.

A solution of the ester **7d** (42.1 g, 0.142 mol) in 100 mL of dry Et_2O was added over 1.5 h to a suspension of $LiAlH_4$ (5.9 g, 0.156 mol) in 400 mL of dry Et_2O with mechanical stirring. The mixture was stirred at reflux temperature for another 1.5 h, cooled, and treated by successive addition of H_2O (6 mL), 15% NaOH (6 mL), and H_2O (18 mL). The granular solids were collected and washed with Et_2O (100 mL). The Et_2O filtrates and washes were combined and dried ($MgSO_4$), and the Et_2O was removed in vacuo. Fractional distillation afforded 25.2 g (84%) of **10d**: bp 163–165 °C (0.1 mm); n^{25}_D 1.5705; IR (neat) 3250–3350 (OH, br), 3025, 3000, 2900, 2850, 1600, 1500, 1450, 1020–1045, 770, and 700 cm^{-1}. A fraction, bp 164–165 °C (0.1 mm), n^{25}_D 1.5705, was used for an analytical sample. Anal. ($C_{11}H_{16}SO_2$) C, H, S.

General Preparation of 2-(Substituted sulfonyl)-1,4-butanediols 11a–g. The alkylthio diols (Table I) were converted to the corresponding sulfonyl diols by the method of Schultz.[12]

General Preparation of 2-(Substituted sulfonyl)-1,4-bis(methanesulfonoxy)butanes 12a–g. The following example illustrates the procedure.

***dl*-2-(*n*-Butylsulfonyl)-1,4-bis(methanesulfonoxy)butane (12a).** Dry pyridine (56.2 g, 0.712 mol) was added dropwise to a stirred solution of **11a** (37.5 g, 0.178 mol) and CH_3SO_2Cl (40.65 g, 0.356 mol) in CH_2Cl_2 (60 mL). The addition requires 3.5–4 h during which time the temperature is maintained between 20 and 30 °C by cooling when necessary. After standing at 0 °C overnight, the mixture was extracted twice with 2 N HCl. The organic layer was separated, washed with H_2O, 5% $NaHCO_3$, and saturated NaCl solution, and dried ($MgSO_4$). Removal of the solvent in vacuo below 50 °C afforded 61.3 g (93.9%) of a syrup which crystallized on standing. Three recrystallizations from EtOH gave 30.1 g (46%) of **12a**, mp 59.5–61.5 °C. Two additional recrystallizations from EtOH afforded an analytical sample, mp 61–63 °C. Anal. ($C_{10}H_{22}S_3O_8$) C, H, S.

Antitumor Testing. Compound **12b** gave an ED_{50} of 31 μg/mL when tested as an alcoholic solution in the KB in vitro cell culture system. Compounds **12a–f** were assayed by intraperitoneal (ip) injection in BDF_1 mice implanted ip with 10^5 L1210 lymphoid leukemia cells. Evaluation of **12b** and **12g** was by ip injection in BDF_1 (**12b**) and CDF_1 (**12g**) mice that were implanted ip with 10^6 P388 lymphocytic leukemia cells. These compounds were injected ip as suspensions in saline (S), saline with Tween 80 (T), klucel (HPC), or carboxymethylcellulose (CMC). The compound, vehicle, highest dose tested (mg/kg), and number of such doses follow in order. For the L1210 system: **12a**, S, 400, 1; **12b**, HPC, 400, 3; **12c**, CMC, 400, 1; **12d**, S, 400, 1; **12e**, T, 200, 9; **12f**, HPC, 400, 9. For the P388 system: **12b**, HPC, 400, 3; **12g**, HPC, 200, 9. All compounds were inactive at the highest dose tested.

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Synthesis and Some Pharmacological Properties of the 23-Peptide 15-Lysine-secretin-(5–27). Special Role of the Residue in Position 15 in Biological Activity of the Vasoactive Intestinal Polypeptide

Miklos Bodanszky,* Sesha Natarajan,

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

Jerry D. Gardner,

Section on Gastroenterology, Digestive Diseases Branch, National Institutes of Health, Bethesda, Maryland 20014

Gabriel M. Makhlouf,

Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

and Sami I. Said

Dallas V.A. Hospital and the University of Texas Health Science Center, Dallas, Texas 75216. *Received May 8, 1978*

The 23-peptide 15-lysine-secretin-(5–27) [S(5–27)] was synthesized on an insoluble support. The residue in position 15 of secretin, aspartic acid, was replaced by lysine, which occupies that position in the vasoactive intestinal polypeptide (VIP), a member of the secretin family. The resulting analogue showed increased VIP-like activity on smooth muscle preparations and unaltered secretin-like activity on pancreatic juice secretion in the rat. The affinity of the new analogue for high-affinity secretin receptors in acinar cells from guinea pig pancreas was less than that of S(5–27) but was higher than that of S(5–27) for high-affinity VIP receptors in the same cells.

The discovery of a series of gastrointestinal peptide hormones, followed by the elucidation of their amino acid sequences, uncovered patterns of structural similarities between them. Obvious sequence homologies exist between gastrin, cholecystokinin-pancreozymin, and caerulein and between secretin, vasoactive intestinal peptide (VIP), glucagon, and gastric inhibitory peptide (GIP). These homologies favor the concept of hormone families.[1] The similarities between the individual members of these families have received considerable attention. Less attention has been devoted to the study of structural *differences* between related peptides. The differences are no less significant for they seem to be responsible for differences in the biological activities of these peptides. In this paper, we present the results of a study designed to elucidate the role of the amino acid residue which occupies position 15 in secretin and VIP.

An analysis of the homologies in the sequences of the members of the secretin family (Figure 1) reveals a conspicuous difference between secretin and VIP with respect to position 15. This position is occupied by an acidic aspartyl residue in secretin[2] and by lysine, a basic moiety, in VIP.[3] Ion pairs, it might be assumed, would result from the interaction of secretin and its putative receptor. Yet, in secretin the 15-aspartyl residue participates in an intramolecular ion pair,[4] so its similar involvement with a cation-forming site in the receptor seemed unlikely. This did not exclude a role in the hormone-receptor interaction for the corresponding residue in VIP. To find evidence for such a role for the 15-lysine of VIP, we synthesized a secretin analogue with lysine replacing aspartic acid in position 15 of secretin. Because of some complications in the synthetic procedure, which will be discussed below, the C-terminal 23-peptide part of secretin rather than the entire sequence of the hormone was modified. This portion of the molecule, S(5–27), has intrinsic activity[5] and, therefore, its 15-lysine analogue seemed to be suitable for comparisons with the unaltered sequence with respect to binding to receptors. The VIP-like effect of C-terminal sequences of VIP was demonstrated earlier.[6]

The 23-peptide, 15-lysine-S(5–27), was synthesized on an insoluble support.[7] The latter, an aminobenzhydryl resin,[8] was acylated with the *o*-nitrophenyl ester of *tert*-butyloxycarbonyl-L-valine[9] and—after deprotection—the chain was lengthened in a stepwise[10] fashion with *tert*-butyloxycarbonylamino acid active esters. Nitro-L-arginine residues were introduced by activation of the *tert*-butyloxycarbonyl derivative with dicyclohexylcarbodiimide[11] in the presence of 1-hydroxybenzotriazole.[12] While in most cases *o*-nitrophenyl esters which were found suitable in solid-phase synthesis were used,[13] threonine was incorporated as the *N-tert*-butyloxycarbonyl-*O*-benzyl-L-threonine 2,4,5-trichlorophenyl ester.[14] The active ester reactions were catalyzed[15] with 1-hydroxybenzotriazole and were followed by the similarly catalyzed acetylation of any unreacted amine with *p*-nitrophenyl acetate. In the introduction of the first few residues, the recently recommended[16] medium, toluene, was used but in the subsequent steps dimethylformamide seemed to be more advantageous.

After the incorporation of 23 amino acids, the synthesis was continued to the completion of the 27-membered chain of the secretin analogue only with an aliquot of the peptidyl-resin. An attempt to remove the secretin analogue from the resin resulted in a material which, according to the data of amino acid analysis after enzymic degra-

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