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The synthesis and biological activity of 4-[bis(2-chloroethyl)amino]-DL-, L-, and D-phenylalanine amides and peptides

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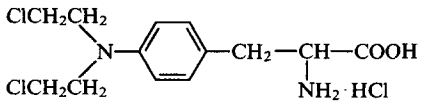
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Abstract. The review is devoted to the synthesis and biological properties of the amides and peptides containing the stereoisomers of 4-[bis(2-chloroethyl)amino]phenylalanine. The approaches to the selection of the structures of the compounds indicated, ensuring an increase in the selectivity of their antitumour activity, are examined. The bibliography includes 131 references.

I. Introduction

Sarcolysine (Sarc) or 4-[bis(2-chloroethyl)amino]-DL-phenylalanine hydrochloride **1** and melphalan (Mel) or 4-[bis(2-chloroethyl)amino]-L-phenylalanine hydrochloride **2**, which are antitumour preparations widely used at the present time, have been synthesised and investigated independently and simultaneously by two groups of investigators in the USSR and England.



1–3

Continuing the idea of L F Larionov concerning the use of essential metabolites, in particular aminoacids, as carriers for the alkylating group, sarcolysine[1] and its enantiomers based on L- and D-phenylalanines have been synthesised in the Soviet Union. The D-enantiomer **3** [medphalan (Med)] was synthesised in England under Bergel's supervision.[2,3]

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On the basis of experimental data, the authors[2,4] established that the L-form **2** is more active than the D-form **3**. This is probably associated with the difference in the transport of the stereoisomers, their chemical transformation in the organism (medphalan is deaminated by D-aminooxidase much faster than melphalan), and the nature of the tumours.[5]

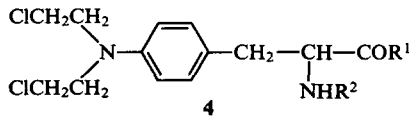
It was established that the transport of melphalan to the tumour cell takes place with the aid of two transferring systems: the L system, which transports leucine, phenylalanine, tryptophan, and valine, and a system similar to ASC, which transports alanine, serine, and cysteine.[6–8] The pharmacokinetics of ^{14}C-labelled sarcolysine and melphalan have been considered in a review[9] and in another communication.[10] Melphalan has been used clinically for the treatment of the same tumours as sarcolysine.[11]

The high activities of sarcolysine and melphalan and their successful clinical application stimulated the development of a new aspect of the chemotherapy of cancer — the synthesis and study of the derivatives of these compounds, as well as the synthesis of the cytotoxic derivatives of other aminoacids. Aminoacids and peptides are known to pass through the membranes of tumour cells 4–5 times more readily than through those of normal cells[12] and there is therefore a possibility of their accumulation in the cancer cell. On this basis, aminoacids and peptides have been frequently used as 'transporters' of alkylating agents.

II. *N*-Acyl derivatives of 4-[bis(2-chloroethyl)-amino]phenylalanine and their esters, amides, and salts

Some information about the chemistry of alkylating preparations, including the chemistry of sarcolysine peptides, has been surveyed in earlier reviews[13,14] and in the review of Giller et al.[12] Since sarcolysine has a toxic side effect in relation to hematopoiesis, attempts were made to increase the selectivity of its transport into tumour cells by synthesising a wide variety of *N*-acyl derivatives; esters, amides, and hydrazides. A large series of derivatives **4**, synthesised after sarcolysine had been obtained, have been described in a review.[5]

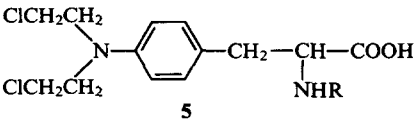
A series of compounds with other aliphatic and aromatic acyl groups as well as urethane and sulfonamide analogues of sarcolysine **5** were obtained later.[15]



4

R^1 = OH, OCH_3, OC_2H_5, $OCH_2C_6H_5$, OC_3H_7-iso, NH_2, NHC_2H_5, NHC_6H_{11}-cyclo, $NHNH_2$ etc.;

R^2 = H, CHO, CH_3CO, CH_3CH_2CO, $CH_3(CH_2)_2CO$, $CH_3(CH_2)_3CO$, $CH_3(CH_2)_{14}CO$, $CH_3(CH_2)_{16}CO$, $HOOC(CH_2)_2CO$, $ClCH_2CO$, ICH_2CO, *p*-$(ClCH_2CH_2)_2NC_6H_4CH_2CO$, nicotinoyl, tetracyclinylmethylene, hydroxytetracyclinylmethylene, etc.

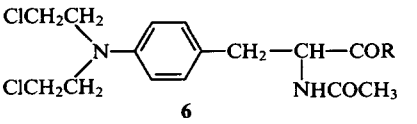


5

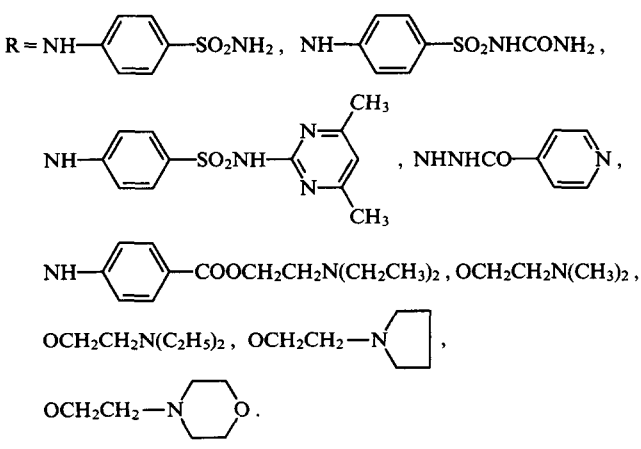
R = CH_3CO, $C_5H_{11}CO$, $C_8H_{17}CO$, $C_{11}H_{23}CO$, $C_6H_5CH_2CO$, C_6H_5CO, *sec*-C_4H_9OCO, C_6H_5OCO, *p*-$CH_3C_6H_4SO_2$.

The compounds were used in biological tests in the form of salts with ethanolamine. They are all characterised by a lower toxicity than sarcolysine. *N*-Acetylsarcolysine **5** (R = CH_3CO) possesses the highest antitumour activity. There is no clear-cut correlation between the physicochemical and biological properties of the compounds. The capacity for metabolic transformations is apparently very important. It may be that the antitumour activity is shown to a greater extent the easier the deacylation of the preparation in vivo.

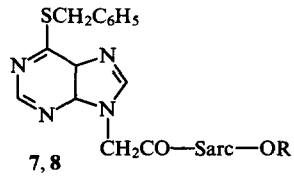
In order to investigate the antitumour properties, a large number of sarcolysine amides were synthesised, for example the *N*-acetylsarcolysine derivative **6** containing streptocide, urosulfan, sulfadimesine, tubazid, and novocaine residues, as well as the dialkylaminoalkyl esters of *N*-acetylsarcolysine.[16]



6



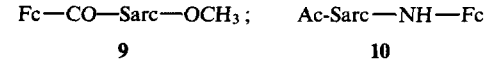
The 6-benzylthio-9*H*-purin-9-ylacetic acid residue was used as the agent for the transfer of sarcolysine into the tumour cell. The derivative **7** was obtained by alkylating 6-benzylthiopurine with the ethyl ester of *N*-(bromoacetyl)sarcolysine. The alkaline hydrolysis of the derivative **7** leads to compound **8**.[17]



7, 8

R = C_2H_5 (**7**), H (**8**).

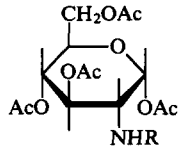
In order to diminish the hematotoxic activity of sarcolysine caused by the introduction of the ferrocene fragment, the methyl ester of N^{α}-ferrocenoylsarcolysine **9** and the ferrocenylamide of *N*-acetylsarcolysine **10** were synthesised.[18]



9 **10**

Fc = ferrocenyl.

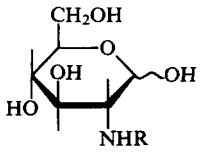
The combination of the biologically active carrier of the cytotoxic group and of the active principle of the latent activity was used in the synthesis of the 1,3,4,6-tetraacetyl-2-amino-2-deoxy-D-glucopyranose derivatives **11** and **12** of *N*-acetylsarcolysine.[19]



11, 12

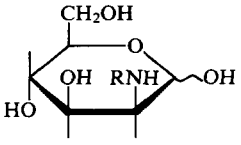
R = Ac-Sarc (**11**), Ac-Sarc – DL-Val (**12**).

Compounds **11** and **12** are readily soluble in water, are less toxic, and exhibit a somewhat higher antitumour activity than sarcolysine. A series of 2-amino-2-deoxy-D-glucopyranose derivatives **13**–**16** and the 2-amino-2-deoxy-D-mannose derivative **17**, in which the amino-group is acetylated by *N*-acetylsarcolysine or a peptide containing it, have been synthesised.[20] Compounds **13**–**17** possess a weak antitumour activity.



13–16

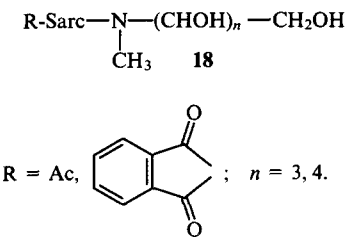
R = Ac-Sarc (**13**),
Ac-Sarc – Val (**14**),
Ac-Sarc – Leu (**15**),
Ac-Sarc – Gly – Gly (**16**).



17

R = Ac-Sarc (**17**).

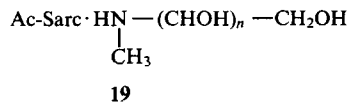
The sarcolysine derivatives of polyhydric 1-methylamino-1-deoxy-alcohols **18** (the 1-methylamino-1-deoxy-derivatives of D-glucitol, D-galactitol, D-mannitol, and L-arabitol) were obtained by the anhydride method or the mixed anhydride method.[20,21] A relation between the structures of the compounds and their biological activities was established. The highest activity was shown by the D-galactitol and D-mannitol derivatives. The spectrum of the antitumour activity of compound **18** resembles that of sarcolysine. In contrast to sarcolysine, all the substances exhibited a minimal effectiveness in the treatment of leukaemia and the highest in the treatment of the MORS-406 plasmacytoma.



18

R = Ac, ; n = 3, 4.

In order to make the compounds soluble in water, the salts of *N*-acetylsarcolysine with the amino-derivatives **19** of the polyhydric alcohols listed above were obtained.[22]

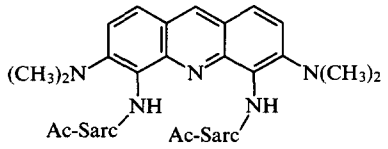


19

n = 3, 4.

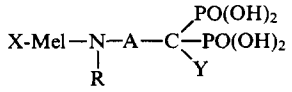
The acetyl derivatives showed a low toxicity and exhibited the highest antitumour activity towards MORS-406 plasmacytoma.

The varied biological activities of the disubstituted 3,6-acridines served as a basis for the synthesis of compound **20**.[23]

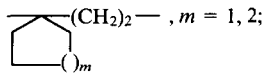


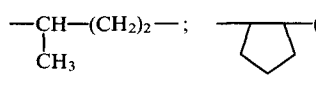
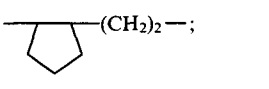
20

In view of the tropism of the 1,1-diphosphonic acid derivatives in relation to bone tissue, a series of melphalan amides **21**, containing two phosphonic acid residues, were synthesised. They exhibit an antitumour activity towards certain tumours.[24]



21

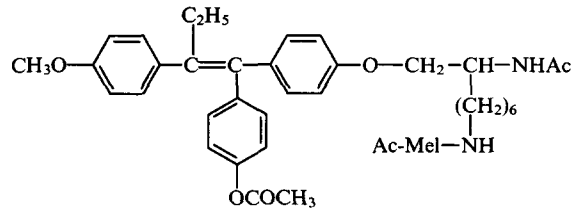
A = $-(CH_2)_n-$, n = 2–5;  , m = 1, 2;

 ; 

R = CH_3, H; X = HCO, $(CH_3)_3COCO$, H; Y = OH, H.

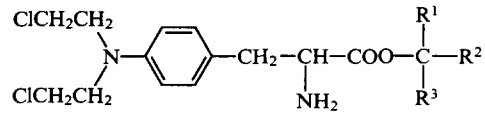
The above acids as well as their tetraethyl esters and sodium salts can be used in the treatment of dense tumours such as multiple myeloma, osteosarcoma, the mammary gland carcinoma, the carcinoma of the ovaries, leukaemia, and lymphomas. The substances are characterised by a high therapeutic index, a low toxicity, and a ready solubility in water, which permits their parenteral and oral administration.

Acetylmelphalan was used, among other cytostatic agents, for the synthesis of antitumour compounds (for example compound **22**) with an affinity for steroid hormone receptors.[25]



22

The antitumour activities and toxicities of the lower esters **23** differ little from those of sarcolysine.[5] The *tert*-butyl esters of sarcolysine **23** ($R^1 = R^2 = R^3 = CH_3$) have been used as therapeutic agents to prevent the occurrence of metastases in the brain.[26]



23

R^1–R^3 = H, Cl, Br, F, I, Alk(C_1–C_3).

One of the groups $R^1 = R^3$ may be a haloalkyl group.

III. The di- and tripeptides derived from the stereoisomers of 4-[bis(2-chloroethyl)amino]-phenylalanine

The procedure used in the synthesis of the 4-[bis(2-chloroethyl)-amino]phenylalanine peptides proved extremely fruitful in increasing the selectivity of the antitumour activity of this compound†. By virtue of the ready availability of sarcolysine, numerous studies have been carried out on the synthesis of peptides based on it with both optically active and racemic aminoacids (mixtures of stereoisomers were then obtained). In the early studies, the content of the stereoisomers in the mixtures was not determined, although interesting results were in fact obtained in the study of their biological activity. The development of methods of synthesis of sarcolysine-containing peptides has been justified objectively, because in the synthesis of potential biologically active compounds a mixture of stereoisomers was frequently isolated initially and then, when a high physiological activity had been discovered, the mixture was separated into the individual stereoisomers. The methods for the formation of the peptide bond, the selective removal of the protecting group, etc. developed for racemic mixtures, have not lost their importance to this day.

A large number of peptides derived from sarcolysine, in which the latter is the N- or C-terminal aminoacid or is present in the middle of the chain, have been synthesised and investigated.[5] Peptides with melphalan have also been described in the review of Rasteikene et al.[5]

A large series of studies on the synthesis of dipeptides, the preparation of individual diastereoisomers, the analysis of their diastereoisomeric purity, and the study of the relation between the biological activity of the compounds and their structure have been carried out under the supervision of E N Shkodinskaya. Dipeptides with *N*-acetylsarcolysine at the N-end occupy a special place among the sarcolysine peptides. It was found that the *N*-acetylsarcolysine dipeptides exhibit a high antitumour activity and at the same time act more selectively than sarcolysine or its dipeptides with a free, formylated, or benzoylated amino-group. The first peptide synthesised and investigated — the ethyl ester of *N*-acetylsarcolysyl-DL-valine **24** (asalin),[27] which has

† With the exception of specially stipulated instances and compounds **1** and **3**, all the aminoacids belong to the L-series.

found an application in practical oncology, was obtained by the dicyclohexylcarbodiimide (DCHC) method.

Ac-Sarc–DL-Val-OC_2H_5 (**24**),
Ac-Sarc–Phe-OC_2H_5 (**25**),
Ac-Sarc–DL-Met-OC_2H_5 (**26**),
Ac-Sarc–Met-OC_2H_5 (**27**),
Ac-Sarc–DL-Trp-OC_2H_5 (**28**),
Ac-Sarc–DL-Ser-OC_2H_5 (**29**),
Ac-Sarc–DL-Asp$(OC_2H_5)_2$ (**30**),
Ac-Sarc–Asp-$(OC_2H_5)_2$ (**31**),
Ac-Sarc–DL-Asp$(OCH_2C_6H_5)_2$ (**32**),
Ac-Sarc–DL-Asp$(OH)_2$ (**33**).

The ethyl esters of *N*-acetylsarcolysine peptides with both racemic aminoacids (methionine, cysteine, phenylalanine, and alanine)[27] and with optically active aminoacids [L-phenylalanine (asaphan **25**), L-methionine (asamet **27**)] were synthesised similarly. The syntheses of a series of *N*-acetylsarcolysine peptides by the mixed anhydride method using the chloride of isovaleric acid have been described.[28] The dipeptides **24–33** with DL-valine, L-phenylalanine, DL- and L-methionine, DL-serine, and L- and DL-aspartic acids have been obtained by this method. Subsequently asalin, asaphan, asamet, asaley (the ethyl ester of *N*-acetylsarcolysyl-L-leucine) and astyron (the ethyl ester of *N*-acetylsarcolysyl-L-tyrosine) became the subject of a more far reaching study in order to introduce the best of them into oncological practice.

A comparative study was made of the toxicity of asalin, asaley, and astyron administered in different ways.[29] Their toxic effects on the organism proved to be similar. Asalin and asaley exhibit a weaker inhibiting effect on the haematopoietic system compared with sarcolysine.[30] The mechanism of the antitumour activities of asalin and asaley[31,32] and their influence on the immune system[33,34] have been investigated comprehensively. The effect of asalin on haematopoiesis,[35–38] the molecular mechanism of antitumour activity,[39–41] the molecular mechanism of its effect on the functional state of mitochondrial membranes in vivo,[42] and its mutagenic activity[42] have been investigated. A method of synthesis of ^{14}C-labelled asaley, containing a label either in the sarcolysine or in the leucine residue, has been developed for the study of its pharmacokinetics.[44]

Asalin has undergone clinical tests and has been recommended for the treatment of myeloma, lymphosarcoma, reticulosarcoma, and ovarian seminoma. Asalin may find the most effective practical application in the treatment of tumours in patients with symptoms of a moderate inhibition of haematopoiesis after radiation therapy and the use of alkylating agents.[11]

In order to obtain water-soluble *N*-acetylsarcolysine dipeptides, a method has been proposed for the deblocking of the carboxy-group by means of the alkaline hydrolysis under mild conditions of their methyl and ethyl esters.[45] The bis(2-chloroethyl)amino-group is unaffected under these conditions. The dipeptides with DL-valine, DL-tryptophan, L-phenylalanine, L-methionine, and L-leucine were synthesised by this method.

The mixed anhydride method employing methyl chlorocarbonate has been used to obtain the sarcolysine-modified carnosine, homocarnosine, and anserine (compounds **34–38**).[46]

Ac-Sarc–β-Ala-OH (**34**),
Ac-Sarc–γ-Abu-OH (**35**),
Ac-Sarc–β-Ala–His-OH (**36**),
Ac-Sarc–β-Ala–N^{im}-Me-His-OH (**37**),
Ac-Sarc–γ-Abu–His-OH (**38**).

The solid-phase synthesis of the peptides **39–41**, analogues of compound **36**, employing *N,N'*-carbonyldiimidazole as the condensing agent, has been described.[47]

HCO-Sarc–β-Ala–His-OCH_3 (**39**),
HCO-Sarc–β-Ala–His-$OCH_2C_6H_5$ (**40**),
HCO-Sarc–β-Ala–His-OH (**41**).

As mentioned above, *N*-acetylsarcolysine dipeptides with optically active aminoacids are mixtures of two diastereoisomers differing in their biological activities. The maximum antitumour activity of each dipeptide is associated with a definite combination of optically active aminoacids. Thus the L,L-isomer is the most active for asaphan, while the L,D- and D,D-forms are the most active for asalin.[13] The preparation of optically active dipeptides of *N*-acetyl-4-[bis(2-chloroethyl)amino]phenylalanine was hindered by the low availability of compounds **2** and **3** and the appreciable racemisation of their acetyl derivatives during the peptide synthesis. It was found that the optically active dipeptides can be obtained most simply by the interaction of *N*-acetylsarcolysine (the racemic form) with the esters of L- and D-aminoacids with subsequent fractional crystallisation of the optical isomers.[13]

For example, the methods for the isolation and the properties of the enantiomers and of the diastereoisomeric mixtures of asalin **24** have been described.[48] The diastereoisomeric racemates were separated chromatographically into the racemate I (compounds **42** and **43**) and the racemate II (compounds **44** and **45**).

Ac-L-Sarc–L-Val-OC_2H_5 (**42**),
Ac-D-Sarc–D-Val-OC_2H_5 (**43**),
Ac-D-Sarc–L-Val-OC_2H_5 (**44**),
Ac-L-Sarc–D-Val-OC_2H_5 (**45**).

The optically active dipeptides **42–45** were isolated by chromatographic separation of the diastereoisomeric peptides synthesised by the reaction of *N*-acetylsarcolysine and the ethyl esters of L- and D-valine. The asalin specified in the pharmacopoeia consists mainly of the racemate II with an admixture of 0.8% of the racemate I. The racemate II proved to have the highest antitumour activity and the highest toxicity of the two racemic diastereoisomeric mixtures.

In order to investigate the influence of the stereoconfiguration of the aminoacids and of the protection of the amino-group on the antitumour properties of the diastereoisomers, the sarcolysylleucine diastereoisomers **46–57** were synthesised (from the aminoacid enantiomers).[49] These peptides contain sarcolysine with a free or formylated amino-group, the C-end being protected by an ester or remaining free. The starting materials employed were the enantiomers of *N*-formylsarcolysine and the *p*-nitrobenzyl ester of leucine. These were condensed with the aid of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, which prevents racemisation.

R^1-L-Sarc—L-Leu—OR^2

46–48

46: R^1 = CHO, R^2 = $CH_2C_6H_4NO_2$.
47: R^1 = CHO, R^2 = H.
48: R^1 = H, R^2 = H.

R^1-L-Sarc—D-Leu—OR^2

49–51

49: R^1 = CHO, R^2 = $CH_2C_6H_4NO_2$.
50: R^1 = CHO, R^2 = H.
51: R^1 = H, R^2 = H.

R^1-D-Sarc—D-Leu—OR^2

52–54

52: R^1 = CHO, R^2 = $CH_2C_6H_4NO_2$.
53: R^1 = CHO, R^2 = H.
54: R^1 = H, R^2 = H.

R^1-D-Sarc—L-Leu—OR^2

55–57

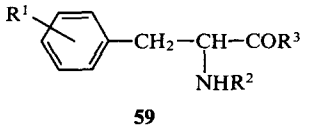
55: R^1 = CHO, R^2 = $CH_2C_6H_4NO_2$.
56: R^1 = CHO, R^2 = H.
57: R^1 = H, R^2 = H.

Dependent on the method of synthesis, isolation, and purification of the *N*-acetylsarcolysine dipeptides with optically active aminoacids, the products are dipeptides with different contents of the diastereoisomers, which is not always reflected in properties such as the melting point and the specific rotation but exerts an appreciable influence on the biological activity. A method has been developed for the quantitative determination of the diastereoisomers of *N*-acetylsarcolysine dipeptides,[50] which involves the chromatographic separation of the diastereoisomers on Silufol UV-254 plate or their preparative separation on silica gel plates with subsequent extraction of the diastereoisomer bands with alcohol and spectrophotometric measurements on the resulting solutions.

The study of the biological activity of the melphalan dipeptide with L-glutamic acid Ac-Mel-Glu(ONa)$_2$ (compound **58**) established that this compound possesses radiation protective properties.[51]

In the further consideration of the studies devoted to the synthesis of peptides modified by 4-[bis(2-chloroethyl)amino]-phenylalanine stereoisomers, special attention will not be given to the methods of formation of the peptide bond because all the peptides were obtained by methods widely known in peptide chemistry with protecting groups constantly employed in amino-acid chemistry and in accordance with schemes which the authors regarded as optimal. In most cases, sarcolysine or its enantiomers were attached to the peptide chain in the last step, because of the presence in the molecule of the labile nitrogen-containing nitrogen mustard group.

The studies of Italian workers headed by Barbieri,[52-54] who synthesised cytotoxic peptides with a postulated dual mechanism of action — alkylation and metabolic inhibition — merit attention. Peptides of melphalan and of its *meta*- and *ortho*-[bis-(2-chloroethyl)amino]-analogues with natural aminoacids (glycine, glutamic acid, lysine, histidine, arginine, phenylalanine, methionine, etc.) or their antagonists (norvaline, norleuceine, ethionine, methylmethionine, methylphenylalanine, *p*-fluorophenylalanine, β-2-thienylalanine, etc.) having the general formula **59** were obtained.

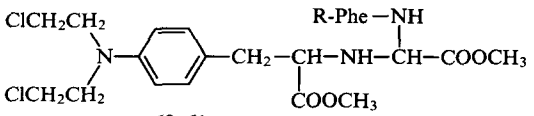


59

R^1 = $N(CH_2CH_2Cl)_2$; R^2 = H, $COOCH_2C_6H_5$, aminoacid residue; R^3 = OH, OAlk, aminoacid residue.

The chemotherapeutic activities of compounds **59** as well as the dipeptides listed above depend significantly on their stereo-configuration and may vary within wide limits. The effect of certain cytotoxic dipeptides on oxidative phosphorylation, etc. processes was studied in the same investigations using experimental strains. Data were obtained confirming the different effects of certain peptides on the enzyme systems of tumours and on the analogous systems in normal tissues.

In order to achieve the selective inhibition of pyruvate kinase in tumour cells, compounds **60** and **61** were synthesised. In these substances, the melphalan residue is attached via an amino-group to the α-carbon atom of the glycine in the phenylalanylglycine molecule.[55]



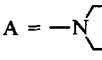
60, 61

R = $C_6H_5CH_2OCO$ (**60**), H·3HCl (**61**).

The antitumour activity of the conjugate **61** proved to be higher in experiments on animals and its acute toxicity was found to be lower than the corresponding properties of melphalan.

Melphalan-containing peptides **62-81**, which are 1,1-diphosphonic acid derivatives, were obtained.[56] These are analogues of the corresponding amides **21** incorporating two phosphonic acid residues.

Mel-Ala-$NH(CH_2)_3C(OH)[PO(OH)_2]_2$ (**62**),
Mel-D-Ala-$NH(CH_2)_3C(OH)[PO(OH)_2]_2$ (**63**),
Mel-Ala-$NH(CH_2)_4C(OH)[PO(OH)_2]_2$ (**64**),
Mel-D-Ala-$NH(CH_2)_4C(OH)[PO(OH)_2]_2$ (**65**),
Mel-Gly-$NH(CH_2)_3C(OH)[PO(OH)_2]_2$ (**66**),
Mel-Gly-$NH(CH_2)_4C(OH)[PO(OH)_2]_2$ (**67**),
Mel-Gly-$N(CH_3)(CH_2)_3C(OH)[PO(OH)_2]_2$ (**68**),
Mel-Pro-$NH(CH_2)_2C(OH)[PO(OH)_2]_2$ (**69**),
Mel-Pro-$NH(CH_2)_4C(OH)[PO(OH)_2]_2$ (**70**),
Mel-γ-Glu-$NH(CH_2)_4C(OH)[PO(OH)_2]_2$ (**71**),
Mel-γ-Glu-$NH(CH_2)_3C(OH)[PO(OH)_2]_2$ (**72**),
Mel-Ala-A-$(CH_2)_2C(OH)[PO(OH)_2]_2$ (**73**),
Mel-Ala-A-$(CH_2)_3C(OH)[PO(OH_2]_2$ (**74**),
Mel-Leu-$NH(CH_2)_2C(OH)[PO(OH)_2]_2$ (**75**),
Mel-Sarc-$NH(CH_2)_3C(OH)[PO(OH)_2]_2$ (**76**),
Mel-Val-$NH(CH_2)_3C(OH)[PO(OH)_2]_2$ (**77**),
Mel-Gly-Gly-$NH(CH_2)_4C(OH)[PO(OH)_2]_2$ (**78**),
Mel-Ala-Gly-$NH(CH_2)_3C(OH)[PO(OH)_2]_2$ (**79**),
Mel-Gly-Gly-Gly-$NH(CH_2)_3C(OH)[PO(OH)_2]_2$ (**80**),
Mel-Ala-Ala-$NH(CH_2)_4C(OH)[PO(OH)_2]_2$ (**81**)

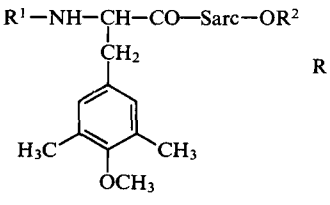
A = .

It was established that compounds **62-81** have antitumour and antimetastatic properties and also exhibit a high inhibiting activity on the growth of nucleic acids in experiments in vitro and an appreciable antitumour activity in vivo. It is believed that these compounds can be used in the treatment of dense tumours: multiple myeloma, osteosarcoma, the metastases of the bone and mammary gland carcinomas, ovarian and testicular carcinomas, as well as various types of lymphomas and leukaemias.

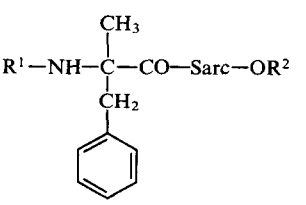
IV. 4-[Bis(2-chloroethyl)amino]phenylalanine peptides with nonprotein aminoacids

The synthetic sarcolysine peptides with protein aminoacids possess a high antitumour activity, but the latter is attained when they are administered in maximum tolerable or toxic doses. The inadequately selective activity of these preparations leads to serious damage to mucous membranes and to the breakdown of haematopoietic and metabolic processes. The hypothesis was put forward that the use of nonprotein aminoacids and the presence of anomalous peptide bonds would make it possible to alter the physiological properties of such peptides.

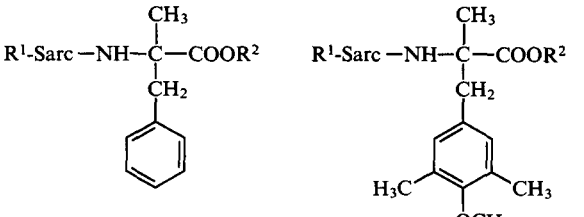
For this purpose, sarcolysine dipeptides with structural analogues of natural aminoacids — 4-methoxy-3,5-dimethylphenylalanine and α-methylphenylalanine (compounds **82-89**) — were synthesised.[57] Dicyclohexylcarbodiimide was used in the condensation stage and the protecting groups were removed by hydrogenolysis.



82, 83



84, 85



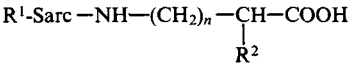
86, 87 **88, 89**

R^1 = HCO, R^2 = $CH_2C_6H_5$ (**82, 84, 86, 88**);
R^1 = R^2 = H (**83, 85, 87, 89**).

The antitumour activities and toxicities of compounds **82–89** were investigated. As in the case of dipeptides with protein aminoacids, the antitumour activities of the diastereoisomers proved to be various.

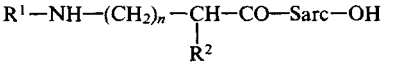
A series of experiments were carried out on the synthesis of peptides containing DL-α-aminobutyric acid (compounds **90, 93, 96,** and **99**), DL-β-aminobutyric acid (compounds **91, 94, 97,** and **100**), and DL-γ-aminobutyric acid (compounds **92, 95, 98,** and **101**).[58–60]

In relation to these compounds, an attempt was made to establish the rules governing the variation of toxicity and antitumour activity, depending on the position of the sarcolysine residue in the peptide, the structure of the second aminoacid, and the presence of the formyl protecting group at the N-terminal aminoacid group.[61–64]



90–95

90: R^1 = H, R^2 = C_2H_5, n = 0; **91**: R^1 = H, R^2 = CH_3, n = 1;
92: R^1 = H, R^2 = H, n = 2; **93**: R^1 = HCO, R^2 = C_2H_5, n = 0;
94: R^1 = HCO, R^2 = CH_3, n = 1; **95**: R^1 = HCO, R^2 = H, n = 2.



96–101

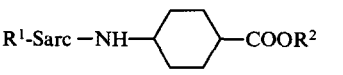
96: R^1 = H, R^2 = C_2H_5, n = 0; **97**: R^1 = H, R^2 = CH_3, n = 1;
98: R^1 = H, R^2 = H, n = 2; **99**: R^1 = HCO, R^2 = C_2H_5, n = 0;
100: R^1 = HCO, R^2 = CH_3, n = 1; **101**: R^1 = HCO, R^2 = H, n = 2.

It was found that the toxicity of the peptides with N-terminal sarcolysine is independent of the position of the amino-group in the C-terminal aminoacid. The toxicity of the peptides with C-terminal sarcolysine is reduced as the methylene bridge becomes longer. In the *N*-formylpeptides, the toxicity diminishes already in the presence of one additional CH_2 group. The peptides containing α-aminobutyric acid have the strongest antitumour activity. In terms of the spectrum of antitumour activity, compounds **90–101** differ little.

The mutagenic and inactivating activities of the peptides **90, 92–95, 96,** and **98–101** in the form of their sodium salts on bacteriophage systems in experiments in vitro have been investigated.[65] The compounds containing sarcolysine at the C-end proved to be more toxic, while those with sarcolysine at the N-end were less toxic. The presence of the formyl group in the peptide does not alter the toxicity of the compound in relation to the bacteriophage.

The study of the mutagenic activities of compounds **90, 92–95, 96,** and **98–101** showed that a change in the structure of the second aminoacid in the sarcolysine peptide molecule alters only slightly the effectiveness of the mutagenic activity. A correlation between the structural changes in the second aminoacid and the mutagenic and inactivating activities of the peptide was not found in the given series of compounds.

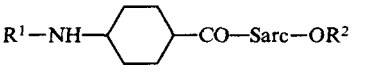
In the course of the continuation of studies designed to establish the possible relations between the toxicological and antitumour characteristics, on the one hand, and the characteristic features of the structure of the sarcolysine peptides, on the other, a series of compounds **102–113**, containing *cis*- and *trans*-4-aminocyclohexanecarboxylic acids (4-ACHCA), were obtained.[66]



102–109

cis	*trans*
102: R^1 = CHO, R^2 = C_2H_5;	**103**: R^1 = CHO, R^2 = C_2H_5;
104: R^1 = H·HCl, R^2 = C_2H_5;	**105**: R^1 = H·HCl, R^2 = C_2H_5;
106: R^1 = $C_6H_5CH_2OCO$, R^2 = $C_6H_5CH_2$;	**107**: R^1 = $C_6H_5CH_2OCO$, R^2 = $C_6H_5CH_2$;
108: R^1 = H, R^2 = H;	**109**: R^1 = H, R^2 = H.

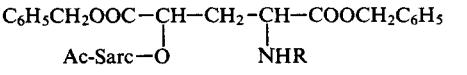
The 4-ACHCA derivatives containing N- and C-terminal sarcolysine residues with both formylated and unsubstituted amino-groups are less toxic compared with the initial sarcolysine. The compounds with a free carboxy-group in 4-ACHCA are less toxic compared with the corresponding esters. All the compounds exhibit a high antitumour activity. The derivatives containing amino- and carboxy-groups in the *trans*-positions are less toxic and have a more pronounced antitumour activity compared with the analogous *cis*-4-ACHCA derivatives.[67]



110–113

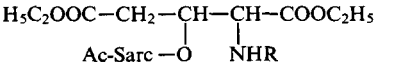
cis	*trans*
110: R^1 = $C_6H_5CH_2OCO$, R^2 = $C_6H_5CH_2$;	**112**: R^1 = $C_6H_5CH_2OCO$, R^2 = $C_6H_5CH_2$;
111: R^1 = H, R^2 = H;	**113**: R^1 = H, R^2 = H.

The importance of the stereoconfiguration of the 'carrier' of the alkylating compounds has been demonstrated in relation to cytotoxic derivatives of 3- and 4-hydroxy-DL-glutamic acids. The acids are capable of inhibiting a series of enzymes involved in the metabolism of glutamic acid.[68] Modification with sarcolysine can make the inhibition irreversible. By varying the method by which *N*-acetylsarcolysine is attached to the above compounds, i.e. by the formation of ester or peptide bonds with the *threo*- or *erythro*-diastereoisomers of 3- and 4-hydroxyglutamic acids, the derivatives **114–122** were obtained.[69–71]

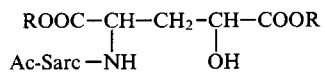


114–116

cis: R = $COOCH_2C_6H_5$ (**114**); H·HCl (**115**)
trans: R = H·HCl (**116**).



threo-**117**, *erythro*-**118**.



119–122

threo: R = $CH_2C_6H_5$ (**119**); H (**120**).

erythro: R = $CH_2C_6H_5$ (**121**); H (**122**).

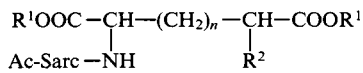
The stereoisomers of the esters **114–118** and the peptides **120–122** exhibited low antitumour activities and an appreciable toxicity, but differed both as regards the magnitude of the antitumour activity and the spectrum of the latter.[69, 71] This shows that, as in the case of the sarcolysine peptides with protein aminoacids, the configuration of the nonprotein aminoacids entering into the composition of the cytotoxic esters and peptides exerts an appreciable influence on the antitumour activity.

An attempt has been made[72] to investigate the influence of the stereoconfigurations of the individual aminoacids forming part of the peptide on the antitumour activity of the latter. The stereoisomers of the tripeptides **123–126** were synthesised.

Lys–Med–Glu (**123**), Lys–Mel–Glu (**124**),
Lys–Sarc–*threo*-4-OH-Glu (**125**),
Lys–Sarc–*erythro*-4-OH-Glu (**126**).

Compounds **123–126** are modified fragments of the ribonuclease S-peptides. Furthermore, the tripeptides **124** incorporate, as it were, two dipeptides: Lys–Mel and Mel–Glu, which exhibit a very high antitumour activity on various strains of tumours. However, the results of tests of compounds **123–126** on animals revealed their low antitumour activities, which precluded a conclusion about the influence of aminoacids on the latter.

In order to increase the proteolytic stability and biological activity of the sarcolysine dipeptides, peptides based on homoglutamic and diaminoadipic acids and the diastereoisomers of diaminoglutaric acid (compounds **127–133**) were synthesised.[73]

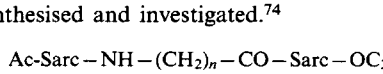


127–133

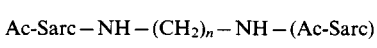
127: $R^1 = C_2H_5$, R^2 = H, n = 2;
128: $R^1 = C_2H_5$, R^2 = Ac-Sarc–NH, n = 2;
129: $R^1 = C_2H_5$, R^2 = Ac-Sarc–NH, *meso*-, n = 1;
130: $R^1 = C_2H_5$, R^2 = Ac-Sarc–NH, *threo*-, n = 1;
131: R^1 = Na, R^2 = H, n = 2;
132: R^1 = Na, R^2 = Ac-Sarc–NH, n = 2;
133: R^1 = Na, R^2 = Ac-Sarc–NH, *meso*-, n = 1.

Despite the increase in the susceptibility to alkylation in experiments in vitro, the introduction of a second sarcolysine residue into the molecules of compounds **128–130**, **132**, and **133** did not lead to an increase in the antitumour activity. This can probably be accounted for by the difficulty of transporting the disarcolysine compound through the membranes of the tumour cell.

The tripeptides of the β-, γ-, and ε-aminoacids **134** containing N- and C-terminal *N*-acetylsarcolysine residues with different numbers of CH_2 groups between them as well as their analogues — *N*-acetylsarcolysine alkylenediamides **135** — were synthesised and investigated.[74]



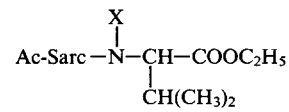
134, n = 2, 3, 5.



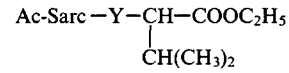
135, n = 2, 3.

Compounds **134** proved to be inactive, while compounds **135** exhibited a moderate activity.

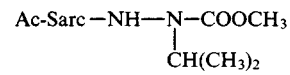
The antitumour preparations asalin[75] and asaphan[76] have been modified. The peptide bond was altered in asalin **24**. The hydrogen in the peptide amide group was replaced by various residues (compounds **136**): the amide group was replaced by ester, hydrazide, or amidoxy-groups (compounds **137**) and DL-valine was replaced by 2-azavaline (compound **138**).



136: X = C_2H_5, OC_2H_5, OH, $NH_2 \cdot HBr$.

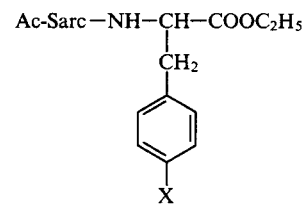


137: Y = O, NHNH, NHO.

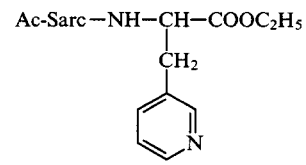


138

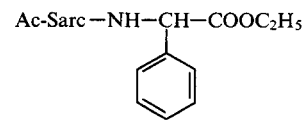
In asaphan, the benzene ring of phenylalanine was modified and its position in the aminoacid was also altered (compounds **139–142**).



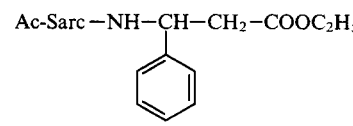
139: X = Cl, NO_2, n-C_3H_7, iso-C_3H_7, n-$C_7H_{15}O$.



140



141

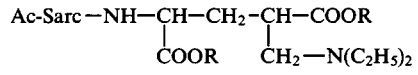


142

The asalin and asaphan analogues **136–142** obtained exhibited towards a series of tumours antiblastic activities similar to and in some cases lower than those of asalin and the ethyl ester of *N*-acetylsarcolysyl-DL-phenylalanine used as controls.

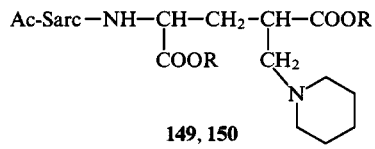
The *N*-acetylsarcolysine dipeptides with the ethyl esters of *N*-methyl-*N*-phenylglycine (compounds **143** and **144**) and of aminohydroxyacids (compounds **145** and **146**)[16] and also with glutamic acid derivatives (compounds **147–150**)[71] have been synthesised.

Ac-Sarc-N(CH_3)$CH_2COOC_2H_5$ (**143**),
Ac-Sarc-N(C_6H_5)$CH_2COOC_2H_5$ (**144**),
Ac-Sarc-NHOCH(C_2H_5)$COOCH_3$ (**145**),
Ac-Sarc-NHOC$H_2COOC_2H_5$ (**146**)



147, 148

R = $CH_2C_6H_5$ (**147**), H (**148**).



149, 150

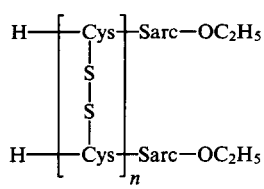
R = $CH_2C_6H_5$ (**149**), H (**150**).

Thus compounds of interest for practical oncology have not so far been discovered among the sarcolysine peptides with nonprotein aminoacids.

V. Polypeptides containing 4-[bis(2-chloroethyl)-amino]phenylalanine

One of the ways of increasing the selectivity of the action of cytostatic substances involves the use of biopolymers, and in the first place polypeptides, for their transport into the tumour cell. Polypeptides have a series of advantages: rapid passage of some of them through the membranes of cancer cells (compared with normal cells) and extensive possibilities for the variation of their composition. The antitumour activities of polypeptide derivatives have been investigated as a function of the nature and configuration of the aminoacid and the length of the peptide chain. A series of homopolypeptidyl derivatives of sarcolysine (compounds **151**–**154**,[77] **155**,[77,78] **156**, and **157**[79]) have been obtained by the polycondensation of the *N*-carboxyanhydrides of various α-aminoacids in the presence of the ethyl ester of sarcolysine. In the case of L-cystine, the derivative **158**, containing disulfide bonds, was synthesised.[80]

$(Val)_n$–Sarc-OC_2H_5 (**151**),
$(D\text{-}Val)_n$–Sarc-OC_2H_5 (**152**),
$(DL\text{-}Nle)_n$–Sarc-OC_2H_5 (**153**),
$(Sar)_n$–Sarc-OC_2H_5 (**154**),
$(Phe)_n$–Sarc-OC_2H_5 (**155**),
$(Lys)_n$–Sarc-OC_2H_5 (**156**),
$(Asp)_n$–Sarc-OC_2H_5 (**157**)



158

The toxicity of compounds **151**–**158** depends on the length of the peptide chain and on the nature of the α-aminoacid constituting the chain. An increase in chain length lowers the toxicity. The polysarcosyl derivatives **154** showed the highest antitumour activity.

The inclusion of an additional aminoacid between the polypeptide chain and sarcolysine alters the activity of the compound (compounds **159**–**165**).[81]

$(DL\text{-}Ala)_n$–Gly–Sarc-OC_2H_5 (**159**),
$(DL\text{-}Ala)_n$–DL-Val–Sarc-OC_2H_5 (**160**),
$(DL\text{-}Ala)_n$–DL-Nle–Sarc-OC_2H_5 (**161**),
$(DL\text{-}Val)_n$–DL-Ala–Sarc-OC_2H_5 (**162**),
$(DL\text{-}Ala)_n$–DL-Nle–Sarc-OC_2H_5 (**163**),
$(Gly)_n$–DL-Ala–Sarc-OC_2H_5 (**164**),
$(DL\text{-}Nle)_n$–Gly–Sarc-OC_2H_5 (**165**).

After the inclusion of an additional aminoacid, the polypeptidyl derivative of which is active, the antitumour activities of the initial homopolypeptides are retained.

Copolypeptides of two types but with the same composition, where one of the two aminoacids entering into the composition of the copolypeptide was introduced between the sarcolysine ethyl ester residue and the polypeptide chain, have been synthesised (compounds **166**–**169**).[81]

$[(DL\text{-}Ala)_{n-1}$–$(DL\text{-}Val)_n]$–DL-Ala–Sarc-OC_2H_5 (**166**),
$[(DL\text{-}Ala)_n$–$(DL\text{-}Val)_{n-1}]$–DL-Val–Sarc-OC_2H_5 (**167**),
$[(Gly)_{n-1}$–$(DL\text{-}Nle)_n]$–Gly–Sarc-OC_2H_5 (**168**),
$[(Gly)_n$–$(DL\text{-}Nle)_{n-1}]$–DL-Nle–Sarc-OC_2H_5 (**169**).

It was established that the antitumour activity of the polypeptide depends on which aminoacid is linked directly to sarcolysine.

A series of copolypeptidyl derivatives of sarcolysine with an irregular structure have been obtained (compounds **170**–**177**[78] and **178**–**184**[79]).

Poly(DL-Ala, DL-Val)–Sarc-OC_2H_5 (**170**),
Poly(Sar, DL-Val)–Sarc-OC_2H_5 (**171**),
Poly(Sar, DL-Nle)–Sarc-OC_2H_5 (**172**),
Poly(Gly, DL-Nle)–Sarc-OC_2H_5 (**173**),
Poly(Gly, DL-Leu)–Sarc-OC_2H_5 (**174**),
Poly(DL-Leu, DL-Nle)–Sarc-OC_2H_5 (**175**),
Poly(DL-Nle, Phe)–Sarc-OC_2H_5 (**176**),
Poly(DL-Nle, DL-Val)–Sarc-OC_2H_5 (**177**),
Poly(Lys, Gly)–Sarc-OC_2H_5 (**178**),
Poly(Lys, DL-Ala)–Sarc-OC_2H_5 (**179**),
Poly(Lys, DL-Val)–Sarc-OC_2H_5 (**180**),
Poly(Lys, DL-Nle)–Sarc-OC_2H_5 (**181**),
Poly(Lys, Phe)–Sarc-OC_2H_5 (**182**),
Poly(Asp, DL-Ala)–Sarc-OC_2H_5 (**183**),
Poly(Asp, DL-Nle)–Sarc-OC_2H_5 (**184**).

Among the copolypeptidyl derivatives, compounds containing glycine or DL-alanine are the most active. It has been shown that both the homopolypeptides and the copolypeptides exhibit a distinct antiblastic activity in relation to tumour strains, but they are largely inferior in their activity to sarcolysine. A number of substances have antitumour activities in relation to certain experimental tumour strains which are superior to that of sarcolysine and at the same time do not exert a general toxic effect on the organism such as that of sarcolysine.[82,83]

The use of conjugates of melphalan with poly-L-aminoacids, in particular with poly-L-lysine (compounds **185** and **186**) and poly-L-glutamic acid (compounds **187** and **188**) as agents transferring the alkylating substances into tumours has been described (Table 1).[84] Poly-L-lysine or poly-L-glutamic acid was condensed with melphalan or its derivative by the DCHC method, after which the protecting groups were removed if necessary.

The conjugates **185**–**188** exhibited in experiments in vitro an alkylating activity amounting to 42%–70% of that of melphalan. Studies of compounds **187** and **188** in experiments on animals established that they are transferred into tumours with the aid of the lymphatic system; this can be used to prevent the occurrence of metastases in lymph nodes.

Table 1. The composition of the conjugates of polyaminoacids with melphalan.

Positions of conjugates	Mel–poly(L-Lys)		Mel–poly(L-Glu)	
	185	**186**	**187**	**188**
Number of aminoacid residues per melphalan molecule	6.7	6.9	23.0	23.1
Number of melphalan molecules per polyaminoacid molecule	83.0	2.3	20.2	4.7

VI. Analogues of natural proteins containing 4-[bis(2-chloroethyl)amino]phenylalanine

In most of the studies quoted above, the combination of aminoacids in the cytotoxic peptides was empirical in character and did not correspond to a definite aminoacid sequence in natural peptides. Studies on the synthesis of analogues of natural proteins containing sarcolysine or its enantiomers are presented below.

Thus, in the search for compounds with a high selectivity in their antitumour activities, the short peptides **189**[85,86] and **190–193**[86] have been synthesised. These are fragments of biologically important proteins such as ribonuclease and histones, because it is known that certain fractions of histones have the ability to inhibit the DNA-dependent synthesis of RNA in experiments in vitro.[87–89] Sarcolysine was introduced into the peptide instead of phenylalanine or alanine or as an additional aminoacid.

Lys–Val–Sarc-OH (**189**),
Lys–Arg–Nle–Sarc-OCH_3 (**190**),
Lys–Sarc-OCH_3 (**191**),
Ala–Lys–Sarc-OCH_3 (**192**),
Ala–Ala–Lys–Sarc-OCH_3 (**193**).

Despite the presence of the labile bis(2-chloroethyl)amino-group, the peptides containing a sarcolysine residue can be obtained by solid-phase synthesis. Thus the sarcolysine-modified glutathione fragment **194** has been obtained on a partially chloromethylated styrene–divinylbenzene copolymer.[90] DCHC was used as the condensing agent. The sarcolysine residue was attached in the last stage.

HCO-Sarc–Cys–Gly-OH · $NH(C_6H_{11})_2$ (**194**)

In the mechanism of the antitumour activity of sarcolysine, two main components have been differentiated: the nuclear component, associated with the breakdown of the structure and functions of DNA and nuclear proteins, and the membrane component, associated with the damage to the internal membranes of mitochondria and the membranes of the endoplasmic reticulum. The second component actually became the basis of the search for substances interacting irreversibly with various membrane receptors. The peptide hormones, acting at extremely low concentrations and exhibiting a high biological activity and selectivity in their action, have become particularly attractive for the creation of new medicinal preparations from this standpoint. Compounds containing sarcolysine or its enantiomers have been synthesised from fragments of hypophyseal hormones such as the luteinising hormone (LH), the adrenocorticotropic hormone (ACTH), and the somatotropic and α-melanocyte-stimulating hormone (α-MSH). Investigations[91] showed that α-MSH is able to bind to plasma membranes of specific types of tumour cells, particularly to the membrane receptors of human melanoma.

A series of studies[92–102] have been devoted to the synthesis of α-MSH fragments (compounds **195–232**) containing sarcolysine or its enantiomers (Table 2, see over) in order to obtain substances with a high selectivity in their antitumour activity.

The stereoisomers of the tetrapeptides **195**, **196**, **198**, and **199** were synthesised from sarcolysine and separated by column chromatography. The protected pentapeptides **216–219** were obtained from them.[92]

Compounds **220–227** were synthesised from the corresponding peptides with N-terminal formylsarcolysine and were then separated chromatographically on silica gel columns and *N*-formylmelphalan and *N*-formylmedphalan were isolated. After the removal of the formyl group, the compounds were condensed with the N-terminal fragment.

In the study of the antitumour activity, it was established that some of the compounds exhibit an antiblastic activity towards a series of transplantable tumours. However, appreciable advantages compared with sarcolysine were not discovered.[101] The compounds with melphalan are more active. The conjugate **232** of α-MSH with melphalan exhibited a greater affinity for receptors than a simple mixture of these compounds.[102] It was established that the replacement of methionine, arginine, or phenylalanine in the initial sequence of α-MSH by melphalan leads to the irreversible binding of the conjugate to the receptor and the prolongation of activity and that on replacement of glycine no inhibition was observed.[96]

It was found that the peptides **214**, **215**, **228**, and **230** exhibit a lower mutagenic activity than melphalan. Presumably, when these compounds are used as antitumour agents, the probability of the appearance of secondary tumours is lower than in the case where melphalan is employed.[99] The peptides **214**, **215**, **228**, and **230** differ also in their toxicity to particular types of cells. They are all less cytotoxic than melphalan.[101]

Attempts have been made recently to increase the selectivity of the action of sarcolysine by attaching it to hypothalamic hormones, the so called releasing factors of releasing-inhibiting factors (according to another nomenclature—liberins and statins), which respectively stimulate or inhibit the liberation of hypohyseal hormones. By regulating the activity of the hypophyseal-adrenal system and steroid genesis, hypothalamic hormones act on hormone-dependent tumours. For example, analogues of the LH-releasing factor and the gonadotropin-releasing factor exhibit an antitumour activity towards experimental tumours.[103] One of the gonadotropin-releasing factor analogues has been used successfully in medical practice in the treatment of hormone-dependent tumours.[104] Melanostatin exhibits an inhibiting activity towards melanoma.[105–107] Hypothalamic hormones are peptides with a relatively small number of aminoacids, which makes their synthesis comparatively simple and easy to carry out.

This stimulated the synthesis and study of analogues of the α-MSH releasing-inhibiting factor (melanostatin)—compounds **233**,[108], **234**,[108–110] **235**,[108, 109] **236–238**,[108] **239**,[108, 110, 111] and **240**.[109, 111]

Pro–Leu–Gly-NH_2 (melanostatin),
Pro–Sarc–Gly-NH_2 (**233**),
Pro–Mel–Gly-NH_2 (**234**),
Pro–Med–Gly-NH_2 (**235**),
HCO-Sarc–Pro–Leu–Gly-NH_2 (**236**),
HCO-Mel–Pro–Leu–Gly-NH_2 (**237**),
HCO-Med–Pro–Leu–Gly-NH_2 (**238**),
Mel–Pro–Leu–Gly-NH_2 (**239**),
Med–Pro–Leu–Gly-NH_2 (**240**).

Table 2. Alkylating analogues of the α-melanocyte-stimulating hormone.

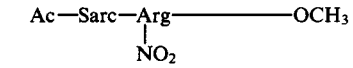
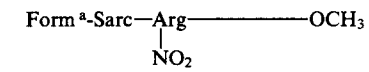
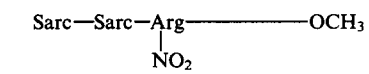
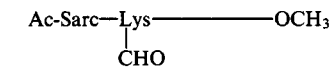
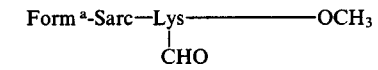
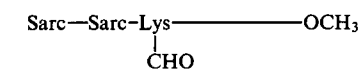
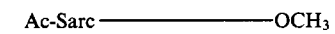
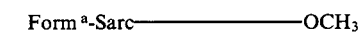
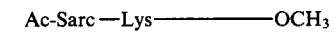
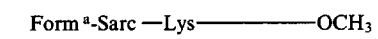
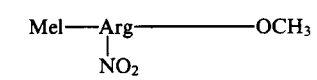
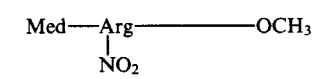
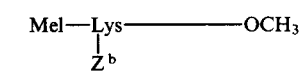
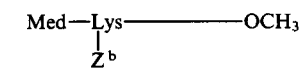
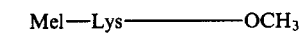
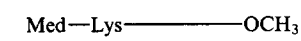
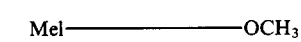
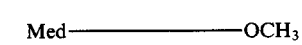
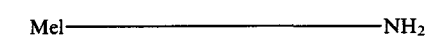
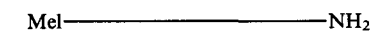
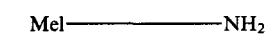
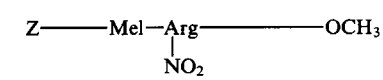
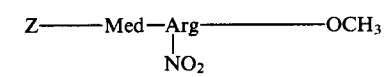
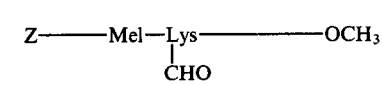
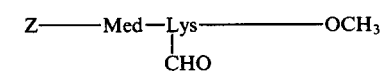
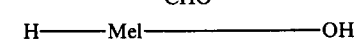
Compound	Formula	Ref.
α-MSH	$\overset{1}{\text{Ac-Ser}}\overset{2}{\text{—Tyr}}\overset{3}{\text{—Ser}}\overset{4}{\text{—Met}}\overset{5}{\text{—Glu}}\overset{6}{\text{—His}}\overset{7}{\text{—Phe}}\overset{8}{\text{—Arg}}\overset{9}{\text{—Trp}}\overset{10}{\text{—Gly}}\overset{11}{\text{—Lys}}\overset{12}{\text{—Pro}}\overset{13}{\text{—Val-NH}_2}$	
195		92
196		92
197		92
198		92
199		92
200		92
201		93
202		93
203		93
204		93
205		94
206		94
207		94
208		94
209		94
210		94
211		95, 96
212		95, 96
213		96
214		96 – 100
215		96 – 100
216		92
217		92
218		92
219		92
220		94, 101

Table 2 (continued).

Compound	Formula	Ref.
α-MSH	1 2 3 4 5 6 7 8 9 10 11 12 13 Ac-Ser—Tyr—Ser—Met—Glu—His—Phe—Arg—Trp—Gly—Lys—Pro—Val-NH_2	
221	H———Med————OH	94, 101
222	H———Mel—Lys————OH	94, 101
223	H———Med—Lys————OH	94, 101
224	H————Mel————OH	94, 101
225	H————Med————OH	94, 101
226	H————Mel—Lys————OH	94, 101
227	H————Med—Lys————OH	94, 101
228	Nle————Mel————OCH_3	96, 98 – 100
229	Nle————Med————OCH_3	96
230	Mel————————OCH_3	96, 98 – 100
231	Mel————phe[c]————OCH_3	96
232	Ac————————Lys————NH_2 Mel	102

Note. Aminoacid residues identical to those in the initial natural peptide are designated by a continuous line.
[a] Form = HCO. [b] Z = $C_6H_5CH_2OCO$. [c] Here and henceforth the aminoacid designation starting with a lower case letter indicates the D-configuration.

Higher antitumour activity of the melphalan-containing melanostatin derivatives compared with the medphalan analogues was discovered.[109] It was noted that the melanostatin fragment in compound **237** protects the organism against the toxic effect of melphalan.[110]

Hypothalamic hormones and their analogues inhibit the secretion of the protein hormones from the hypophysis and the latter inhibit in their turn the secretion of the steroid hormones from the adrenal glands, the levels of which in the hormone-dependent tumours are unduly high. The analogues of hypothalamic hormones can therefore be used to synthesise agents with a high selectivity in their antitumour activity. The synthesis of derivatives of the LH-releasing factor (luliberin) led to the discovery of substances with agonistic and antagonistic properties. These compounds have been used as powerful contraceptives. Luliberin controls the liberation of LH and the follicle-stimulating hormone (FSH), which stimulate in their turn the synthesis of sex hormones. A series of medphalan-containing peptides (compounds **241 – 259**) have been synthesised from luliberin analogues with agonistic and antagonistic properties (Table 3).[112 – 116]

The introduction of hydrophobic D-aminoacids in the 1-, 2-, 3-, and 6- positions and partly in the 5-, and 10-positions in the initial luliberin sequence led to the creation of powerful antagonists. On the other hand, the introduction into the hormone molecules of fragments comprising antitumour preparations known in oncology, such as melphalan or its D-enantiomer, may help to solve the problem of the attainment of a higher selectivity in the antitumour activity. The peptide component of the luliberin analogue makes it possible to increase the selectivity of the action of the alkylating agent as a result of the increased affinity of the peptide fragment for particular receptors on the cell membranes of the hormone-dependent tumours. It has been established that the binding is reversible and no alkylation of the receptors takes place. It is then possible to observe under these conditions a chain of biochemical reactions occurring in the cell.[112]

The peptides have been synthesised mainly by the solid-phase method and only the side chains were attached by the 'classical' method. The compounds obtained were purified with the aid of HPLC. Medphalan was used as an extremely suitable hydrophobic D-aminoacid residue, which can ensure an increase in the biological activity of luliberin analogues.[113]

The medphalan residue was either introduced directly into the peptide chain in the 6-position instead of the glycine residue or it was attached to the ω-amino-groups of lysine and ornithine located in the 6-positions in the luliberin fragments. In a number of cases, a diaminopropionic acid residue was used as the connecting unit for the attachment of two medphalan molecules to lysine or ornithine. The increase in enzyme stability and the formation of active compounds were achieved by the additional incorporation of D-aminoacid residues in the peptide molecules. By estimating the results of the modification of the initial aminoacid sequence in luliberin (the 3- and 6-positions) in compounds **254 – 259**, one can demonstrate that there is a fine balance between the agonistic and antagonistic activities determined by the substituents in these positions. The replacement of D-lysine by D-ornithine in compounds **254** and **255** increased the affinity for the receptors of the mammary gland tumour. Among the luliberin analogues, compounds **241** and **244** exhibited the highest agonistic and antagonistic activities respectively. They exhibited a high affinity for the membrane receptors of the cells of a series of tumours.

Compounds **245 – 253** inhibit the incorporation of [^{3}H]thymidine in DNA in cultures of the mammary gland and prostatic gland tumours and also inhibit the growth of the prostatic gland tumour (in experiments on animals). The peptides **254 – 259** are characterised by a broad spectrum of their affinity for the receptors of the hypophysis and the cell membranes of the mammary gland and prostatic gland tumours.

Table 3. Alkylating analogues of luliberin.

Compound	Formula	Ref.
Luliberin	pGlu—His—Trp—Ser—Tyr—Gly—Leu—Arg—Pro—Gly-NH_2	
241	H—Med—NH_2	113, 114
242	Ac-Pro—Phe(pCl)[a]-trp—Med—NH_2	113
243	Ac-Nal(2)[b]—Phe(pCl)—trp—Arg—Med—ala-NH_2[c]	113
244	Ac-Nal(2)—Phe(pCl)—Pal(3)[d]—Arg—Med—ala-NH_2	113, 114
245	H—lys(ε-Med)—NH_2	112, 115
246	H—lys(ε-$A_2pr^d(Med)_2$)—NH_2	112, 115
247	H—orn(δ-Med)—NH_2	112, 115
248	H—orn(δ-$A_2pr(Med)_2$)—NH_2	112, 115
249	Ac-Nal(2)—Phe(pCl)—trp—lys(ε-Med)—ala-NH_2	112
250	Ac-Nal(2)—Phe(pCl)—trp—Arg—lys(ε-Med)—ala-NH_2	112
251	Ac-Nal(2)—Phe(pCl)—trp—Arg—lys(ε-$A_2pr(Med)_2$)—ala-NH_2	112, 115
252	Ac-Nal(2)—Phe(pCl)—Pal(3)—Arg—lys(ε-Med)—ala-NH_2	112, 115
253	Ac-Nal(2)—Phe(pCl)—Pal(3)—Arg—lys(ε-$A_2pr(Med)_2$)—ala-NH_2	112, 115
254	IA[e]—lys(ε-Med)—NH_2	116
255	IA—orn(δ-Med)—NHC_2H_5	116
256	Ac—lys(ε-Med)—NHC_2H_5	116
257	Ac—orn(δ-Med)—NHC_2H_5	116
258	Ac—lys(ε-Med)—NHC_2H_5	116
259	Ac—lys(ε-Ac-Med)—NHC_2H_5	116

[a] Phe(pCl) = *p*-chloro-D-phenylalanine. [b] Nal(2) = 3-(2-naphthyl)-D-alanine. [c] Pal(3) = 3-(3-pyridyl)-D-alanine.
[d] A_2pr = diaminopropionic acid residue $[-NH-CH_2-CH(NH-)]CO-$. [e] IA = indole-3-acetic acid residue.

A series of analogues of the gonadotropin-releasing hormone (compound **260**), in which melphalan is attached to the transferring molecule via the additional functional group of the aminoacid in the 6-position, have been synthesised.[117] These compounds can be used in veterinary practice to sterilise animals and in medicine in the chemotherapy of hormone-dependent tumours.

pGlu—His—Trp—Ser—Tyr—X(—Y—Mel)—Leu—Arg—Pro—Z

260

X = L- and D-Tyr, L- and D-Orn, L- and D-Glu, L- and D-Asp.
Y = binding element.
Z = $GlyNH_2$, NHC_2H_5, azaglycine amide.

Another series of structurally similar derivatives of the gonadotropin-releasing hormone **261** have been obtained for the same purposes.[118]

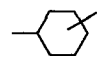
pGlu—His—Trp—Ser—Tyr—D-Lys—Leu—Arg—Y—Z
|
A_1
|
A_2
|
Mel

261

A_1 = —CO—X—N (succinimide ring) ;

A_2 = —CO—CH(NHCOR)—$(CH_2)_n$—S—, n = 1, 2;

R = H, CH_3, C_2H_5;

X = $-(CH_2)_n-$, n = 1–5; $-C_6H_4-$,  , 

Y = Pro, 4-HO-Pro; Z = Gly-NH_2, NHC_2H_5, azaglycine amide.

The analogues of the hypothalamic hormone somatostatin are of special interest for oncology. The concept of the effect of somatostatin derivatives on tumours is analogous to that for luliberin. Somatostatin **262** — a tetradecapeptide — inhibits the liberation of the growth hormone and exhibits a broad spectrum of inhibiting properties.

1 2 3 4 5 6 7 8 9 10 11 12 13 14
Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys
(Cys–Cys)

262

Some of its derivatives have been introduced into medical practice.[119] Conformational analysis and studies of the relation between the structure and function of somatostatin analogues have shown that the sequence necessary for the manifestation of biological activity consists of the β-twisting fragment Phe-Trp-Lys-Thr corresponding to the 7–10 residues of somatostatin.

A group of compounds related to somatostatin (compounds **263–273**) have been synthesised.[120] They differ from one another in the aminoacid residues in the 1-, 3-, 6-, and 8-positions in the peptide chain (Table 4).

Somatostatin retards the growth of tumours in patients with endocrine pancreatic tumours such as insulinoma, glucanoma, and others. However, the hormone is relatively unsuitable for tumour therapy owing to its multiple activity and its brief antisecretary effect. Somatostatin analogues are effective in the treatment of certain types of tumours, including treatment in combination with luliberin analogues.[120] Somatostatin analogues with alkylating fragments can exert both direct and mediated effects on the hormone-dependent tumours, functioning as substances transferring chemotherapeutic agents.

Compounds **263–273** have been synthesised by the solid-phase method and purified by gel filtration on Sephadex or by inverse phase HPLC (all the operations were performed at 4 °C and in the shortest possible time in view of the decomposition of the peptide). They exhibit an inhibiting effect on the liberation of the hypophyseal growth hormone, bind to the membranes of tumour cells, inhibit the incorporation of [^{3}H]thymidine in DNA in cell cultures, and inhibit the growth of particular tumour cells. For use in medical practice, the compounds are obtained in the form of salts: chlorides, phosphates, acetates, etc. or in the form of salts of alkali and alkaline earth metals.[120]

Table 4. Alkylating analogues of somatostatin.[120]

Compound	Formula (1 2 3 4 5 6 7 8)
263	Mel—Cys—Tyr—trp—Lys—Val—Cys—Thr-NH_2
264	Med—Cys—Phe—trp—Lys—Thr—Cys—Thr-NH_2
265	phe—Cys—Mel—trp—Lys—Val—Cys—Thr-NH_2
266	phe—Cys—Tyr—trp—Lys—Val—Cys—Mel-NH_2
267	Mel—phe—Cys—Tyr—trp—Lys—Val—Cys—Thr-NH_2
268	Mel—phe—Cys—Phe—trp—Lys—Thr—Cys—Thr-NH_2
269	Mel—Phe—Cys—Phe—trp—Lys—Thr—Cys—Thr-NH_2
270	Mel—trp—Cys—Phe—trp—Lys—Thr—Cys—Thr-NH_2
271	Mel—phe—Cys—Tyr—trp—Lys—Val—Cys—Trp-NH_2
272	[A2pr(Mel)$_2$]—phe—Cys—Tyr—trp—Lys—Val—Cys—Thr-NH_2
273	Mel—Mel—phe—Cys—Tyr—trp—Lys—Val—Cys—Thr-NH_2

A series of modified derivatives of bombesin have been synthesised as substances interacting irreversibly with the bombesin membrane receptors. Bombesin **274** has been isolated from frog skin and is a tetradecapeptide (Table 5).

The gastrin-releasing peptide, consisting of 27 aminoacid residues and constituting one of the growth factors of the small cell lung carcinoma, is a twin of bombesin in the human organism. By competing with the natural ligand for the receptors, the bombesin antagonists inhibit a series of events leading to abnormal cell proliferation. A large number of melphalan-containing peptides — the bombesin antagonists **275–314** have now been synthesised and patented (Table 5).[121–123] All the compounds have been obtained by the classical methods of peptide chemistry in solutions.

Melphalan has been introduced into both bombesin antagonists and into bombesin analogues with a high affinity for receptors and with agonistic properties. The modified analogues are peptides of the C-terminal component of bombesin obtained by the removal, inversion, or substitution of aminoacids. The analogues in which melphalan is in the 6-position in the bombesin chain exhibit a higher affinity for receptors than the compounds containing melphalan in the 3-position.

Compounds **282**, **285–288**, **293**, **307**, **308**, and **311–314** are less cytotoxic and do not bind to other receptors, for example the epidermal growth factor receptors, which is due to the aminoacid sequence of these compounds.

Gastrin **315** — the gastrointestinal tract hormone — stimulates almost all the functions of the tract: secretion, motor response, and absorption. The amide **316** of the C-terminal tetrapeptide exhibits the full spectrum of physiological activity albeit more weakly than that of the complete molecule.

Table 5. Alkylating analogues of bombesin **274**.

Compound	Formula	Ref.
274	pGlu – Gln – Arg – Leu – Gly – Asn – Gln – Trp – Ala – Val – Gly – His – Leu – Met-NH_2	
275	Mel – Leu-ψ^{a} – NH_2	121
276	Mel – Leu-ψ – Nle-NH_2	121
277	Ac-Mel – Leu-ψ – Nle-NH_2	121
278	Boc[b]-Mel – Leu-ψ – Nle-NH_2	121
279	Ac-Mel – phe – Leu-ψ – Nle-NH_2	121
280	Boc-Mel – phe – Leu-ψ – Nle-NH_2	121
281	Mel – phe – Leu-ψ – Nle-NH_2	122, 123
282	Boc-Mel – OH	
283	Boc-Mel – val – His(Dnp[c]) – NH_2	122, 123
284	Mel – val – His(Dnp) – NH_2	122, 123
285	Mel – His(Dnp) – NH_2	122, 123
286	Boc-Mel – His(Dnp) – NH_2	122, 123
287	Boc-Mel – NH_2	122, 123
288	Mel – NH_2	122, 123
289	Boc-Mel – val – NH_2	122, 123
290	Mel – val – NH_2	122, 123
291	Boc-Mel – val – phe – NH_2	122, 123
292	Mel – val – phe – NH_2	122, 123
293	Boc-Mel – phe – NH_2	122, 123
294	Mel – Thr – □[d] – NH_2	122, 123
295	Mel – ala – NH_2	122, 123
296	Mel – □ – Nle-NH_2	122, 123
297	Mel – NH($(C_2H)_4CH_3$)	122, 123
298	Mel – □ – NH_2	122, 123
299	Mel – □ – NH_2	122, 123
300	Mel – ala – □ – Nle-NH_2	122, 123
301	Mel – ala – □ – NH($C_2H_4C_6H_5$)	122, 123
302	H – □ – <Mel[e]> – □ – Nle-NH_2	122, 123
303	H – Mel – phe – Nle-NH_2	122, 123
304	H – Mel – ala – Nle-NH_2	122, 123
305	Mel – Leu – $NHNH_2$	122, 123
306	Mel – □ – NH_2	122, 123
307	$(Boc)_2$Lys – Mel – □ – NH_2	122, 123
308	Lys – Mel – □ – NH_2	122, 123
309	Mel – Thr – His(Dnp) – NH($(CH_2)_4CH_3$)	122, 123
310	Boc-Mel – Thr – His(Dnp) – NH($(CH_2)_4CH_3$)	122, 123
311	Boc-Mel – □ – NH_2	122, 123
312	Mel – □ – NH_2	122, 123
313	Ac-Lys(Boc) – Mel – □ – NH_2	122, 123
314	Ac-Lys – Mel – □ – NH_2	122, 123

[a] The symbol ψ indicates the reduced CH_2NH peptide bond. [b] Boc = $(CH_3)_3COCO$. [c] Dnp = 2,4-dinitrophenyl.
[d] The symbol □ denotes the absence of the corresponding aminoacid residue in the peptide chain.
[e] The Mel residue is incorporated in the peptide chain between the 5- and 6- positions.

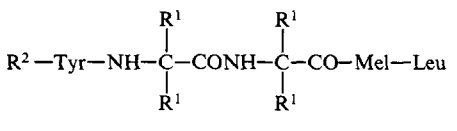
Glu – Gly – Pro – Trp – Leu – $(Glu)_5$ – Ala – Tyr – Gly – – Trp – Met – Asp – Phe-NH_2 (**315**),
Trp – Met – Asp – Phe-NH_2 (**316**),
Boc-Trp – Met – Asp – Sarc-NH_2 (**317**),
Boc-Trp – Met – Asp – Mel-NH_2 (**318**),
Trp – Met – Asp – Sarc-NH_2 (**319**),
Trp – Met – Asp – Med-NH_2 (**320**),
Trp – Met – Asp – Mel-NH_2 (**321**),
Asp – Sarc-NH_2 (**322**),
Asp – Med-NH_2 (**323**),
Asp – Mel-NH_2 (**324**).

In order to create gastrin analogues based on the modification of the C-terminal tetrapeptide **316**, which might act selectively on the tumours of the gastrointestinal tract, a series of derivatives **317**,[124] **318**,[125] and **319–324**[126] have been synthesised. Sarcolysine was used as the starting compound. The mixture of stereoisomers was separated by preparative chromatography. Compounds **319–324** proved to be active in relation to L-1210 lympholeukaemia, the mammary gland adenocarcinoma, the large intestine adenocarcinoma, and sarcoma 37. The peptide **318** has been used in the study of gastrin analogues as inhibitors in a radioimmunoassay system employing antisera, but it exhibited a weak affinity for antibodies and proved physiologically inactive under experimental conditions.

The introduction of various alkylating agents into the peptide chain makes it possible to investigate the characteristics of the interaction of hormones with the receptors and to obtain antagonists to peptide hormones. Melphalan has been used as a label in the study of the affinity of hormones for polypeptide receptors. Derivatives of bradykinin (compound **325**[127]), angiotensin II (compounds **326–328**[128]), and leucine-enkephalin (compounds **329**,[129,130] **330–332**,[130] and **333–336**[131]) have been obtained for this purpose.

Each of the compounds **325–336** synthesised was tested towards the type of receptors to which the corresponding hormone is bound. In particular, enkephalin analogues were studied as irreversible antagonists of the δ-opiate receptor.

Arg – Pro – Pro – Gly – Phe – Ser – Pro – Phe – Arg (bradykinin),
HCO-Sarc – Arg – Pro – Pro – Gly – Phe – – Ser – Pro – Phe – Arg (**325**),
Asn – Arg – Val – Tyr – Ile – His – Pro – Phe (angiotensin II),
Ac-Asn – Arg – Val – Tyr – Ile – His – Pro – Mel (**326**),
Ac-Asn – Arg – Val – Tyr – Ile – His – Pro – Mel-OCH_3 (**327**),
Ac-Asn – Arg – Val – Tyr – Ile – His – Pro – – Mel-$OCH_2C_6H_5$ **328**),
Tyr – Gly – Gly – Phe – Leu (leucine-enkephalin),
Mel – D-Ala – Gly – Phe – Leu-OCH_3 (**329**),
Tyr – D-Ala – Gly – Phe – Leu – Mel-OCH_3 (**330**),
Tyr – D-Ala – Gly – Mel – Leu-OCH_3 (**331**),
Mel – D-Ala – Gly – Mel – Leu-OCH_3 (**332**).



333–336

333: R^1 = H, R^2 = $CH_2CH{=}CH_2$.
334: R^1 = H, R^2 = $CH_2C_6H_5$.
335: R^1 = CH_3, R^2 = $CH_2CH{=}CH_2$.
336: R^1 = CH_3, R^2 = $CH_2C_6H_5$.

Studies on the synthesis and mechanisms of action of polypeptide hormones containing sarcolysine stereoisomers yielded extremely interesting results, the final interpretation of which requires further research.

Thus a large number of peptides, incorporating 4-[bis-(2-chloroethyl)amino]-DL-, L-, and D-phenylalanine and their derivatives have now been synthesised. Sarcolysine dipeptides with protein aminoacids were obtained initially. In many cases, these compounds were of considerable interest for practical oncology. Further search for effective antitumour compounds led to the incorporation of nonprotein aminoacids and aminoacids which are antimetabolites of natural aminoacids in the peptides. Particular attention was then paid to the dependence of the antitumour activity on the stereoconfiguration of the 'carrier' of the alkylating compound.

A series of studies have been devoted to the synthesis of biologically active polymers which are homo- and co-polypeptides modified by sarcolysine and melphalan. The dependence of the antitumour activity on the nature and configuration of the aminoacid and the length of the polypeptide chain in these compounds has been investigated.

Fragments of biologically important proteins, such as histones, enzymes, and hormones, modified by sarcolysine and its enantiomers, were obtained later. The introduction of melphalan or medphalan into fragments of peptide hormones makes it possible to study the mechanism of the interaction of these compounds with the corresponding receptors, which is exceptionally important for the creation of substances with prespecified properties. The presence of an alkylating fragment in the molecule has a mediating effect on the chain of reactions occurring in the cell and makes it possible to obtain compounds with a highly specific activity. These may be not only substances with antitumour activities but also compounds with other types of biological activity.

* * *

The data presented in the review demonstrate the usefulness of the development of methods for the synthesis and study of the peptides of 4-bis(2-chloroethyl)amino]-DL-, -L-, and -D-phenylalanine with natural aminoacids, their synthetic analogues, and fragments of biologically important proteins, because compounds with both high antitumour activity and other types of physiological activity may be discovered among these substances.

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