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Amino Acid and Peptide Derivatives of the Tylosin Family of Macrolide Antibiotics Modified by Aldehyde Function

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Fourteen new functionally active amino acid and peptide derivatives of the antibiotics tylosin, desmycosin, and 5-*O*-mycaminosyltylonolide were synthesized in order to study the interaction of the growing polypeptide chain with the ribosomal tunnel. The conjugation of various amino acids and peptides with a macrolide aldehyde group was carried out by two methods: direct reductive amination with the isolation of the intermediate Schiff bases or through binding via oxime using the preliminarily obtained derivatives of 2-aminooxyacetic acid.

Key words: macrolides, chemical modification, reductive amination, oximes, peptide derivatives, tylosin, desmycosin, 5-O-mycaminosyltylonolide

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INTRODUCTION

The ribosomal tunnel is the least studied functional center of the ribosome [1]. The RT[2] is mainly constructed from nucleotide residues of ribosomal RNA and its entrance part is formed from nucleotides related to ribosomal PTC. During protein synthesis, a segment of the growing polypeptide chain with a length of 25–30 amino acid residues occupies the RT [2], with its conformational state promoting the free displacement of the growing polypeptide chain in the RT, irrespective of its amino acid sequence. On one hand, currently it has only been established that the amino acid residues of the *C*-terminal dipeptide link at the moment of its entrance to the RT are in a β-conformation stabilized by the specific contacts with the RT walls [3], which provides the necessary orientation of the polypeptide chain. On the other hand, recent biochemical studies have shown that the RT is not a passive channel for the passage of the growing peptide: it is a dynamic functional unit in which the specific interactions between the growing peptide and RT walls in turn actively affect the functioning of the ribosome [4–6]. In some cases, such interactions lead to the arrest of the translation that regulates the synthesis of certain proteins [7, 8].

The RNA–protein interactions that take place in the RT and, in particular, the mechanism of the passage and the peculiarities of the conformational state of the growing polypeptide in the RT, remain unknown to date. Synthetic constructs could be used as instruments of the investigation to clear up this problem; on one hand, they could model the elements of the growing polypeptide chain and, on the other hand, could specifically bind with the RT regulatory sites.

Macrolide antibiotics inhibit protein synthesis due to the specific interaction with the high-affinity center, which is formed by the rRNA of the large ribosome subunit and is located inside the RT at a distance of approximately 20 Å from the PTC [9, 10]. Macrolides are bound in this site either with the vacant or the active ribosome at the early stages of translation, when the length of the synthesized peptide chain does not exceed 2–5 amino acid residues in dependence on the size of the macrolide cycle and the length of its oligosaccharide chain [10]. A bound antibiotic blocks the passage of the growing peptide into the channel, which

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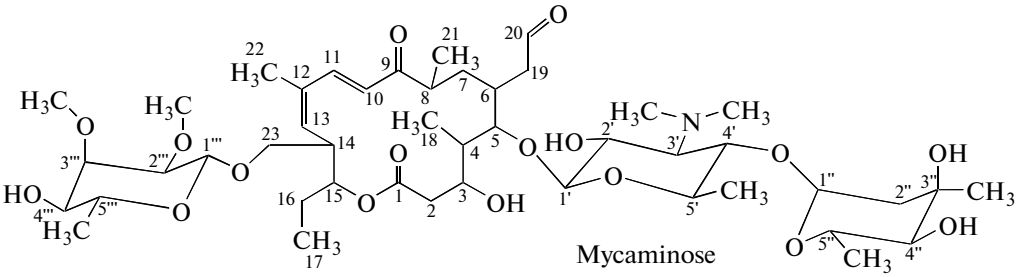
[2] Abbreviations: Aoac, 2-aminooxyacetyl; Des, desmycosin; DIEA, *N,N*-diisopropylethylamine; HBTU, *O*-(benzotriazolyl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazol; OMT, 5-*O*-mycaminosyltylonolide; PTC, peptidyltransferase center; RT, ribosomal tunnel; Tyl, tylosin; and Ual, 3-(uracilyl-1-yl)alanine.

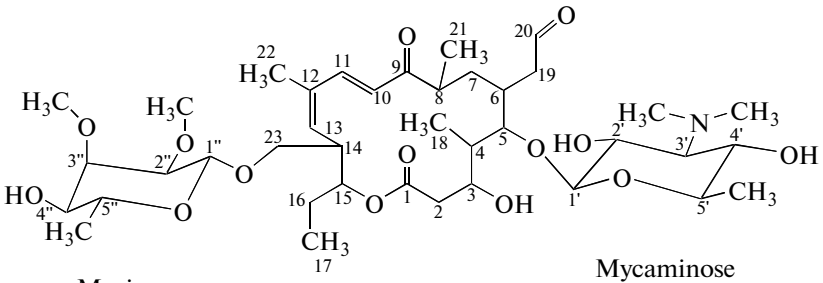
stops the translation and leads to the dissociation of the peptidyl–tRNA–ribosome complex [11, 12].

We previously suggested studying the conformation of the synthetic polypeptide chain in the RT and establishing the chemical nature of the contacts of the polypeptide chains with the RT elements by the synthesis of the model peptide derivatives of macrolide antibiotics with the peptide part mimicking the growing protein chain and the macrolide serving as an anchor for their fixing to a specific RT site [13, 14]. In this case, the main attention was focused on OMT derivatives in which the peptide chain was directed to the exit from the RT [14].

The goal of this work was obtaining the amino acid and oligopeptide conjugates of Tyl, Des, and OMT that differ in their hydrophobicity, charge, and conformational preferences with the use of the most reactive aldehyde group of macrolides.



(I)



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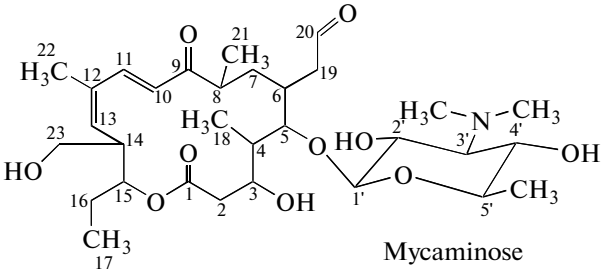


(III)

Tyl [15] (**I**) contains in its structure a two carbohydrate fragment: a disaccharide constructed from mycaminose and mycarose residues at position 5 of the 16-membered lactone cycle and a residue of mycinose at the 14 position. Des (**II**) [16] differs from Tyl by the absence of a mycarose residue, and OMT (**III**) [17] by the absence of mycarose and mycinose residues. All of the macrolides contain a reactive acetaldehyde group at the 6 position.

On one hand, an X-ray analysis of the Tyl complex with a 50S subunit of ribosome [3, 9, 10] indicates that the complex binding to a specific site of the RT leads

to the orientation of the disaccharide substituent at the 5 position of the lactone ring along the PTC (arbitrarily "up" the tunnel); the aldehyde group is also directed up and contacts the adenine base of the nucleotide residue A2062 and, probably, forms a covalent bond with it [10]. On the other hand, it has recently been shown that the A2062 residue in 23S rRNA interacts in a certain manner with the amino acid residues of the regulatory peptide, the synthesis of which in the ribosome results in the stop of translation [18]. In this connection, it was interesting to synthesize hybrid molecules by the conjugation of amino acids, oligopeptides, or their derivatives directly with the aldehyde group of tylosin-series macrolides. Moreover, a number of active Tyl derivatives and its analogues were described in published data. They were obtained by the modification of the aldehyde group, in particular, one of the few commercially available antibiotics, Tyl analogues, tilmycosin (20-deoxo-20-(3,5-dimethyl-1-piperidinyl)desmycosin [19–21].

We supposed that the obtainment of amino acid and peptide derivatives of Tyl, Des, and OMT by the aldehyde group would proceed so that the peptide fragment would be directed from the macrolide ring up the tunnel. To retain the direction, which the synthesized polypeptide chain adapts in the ribosome, it was necessary to attach the model peptides to the macrolide by their *N*-termini. We believed that such constructs might be useful as instruments for studying the mechanism of RT functioning.

RESULTS AND DISCUSSION

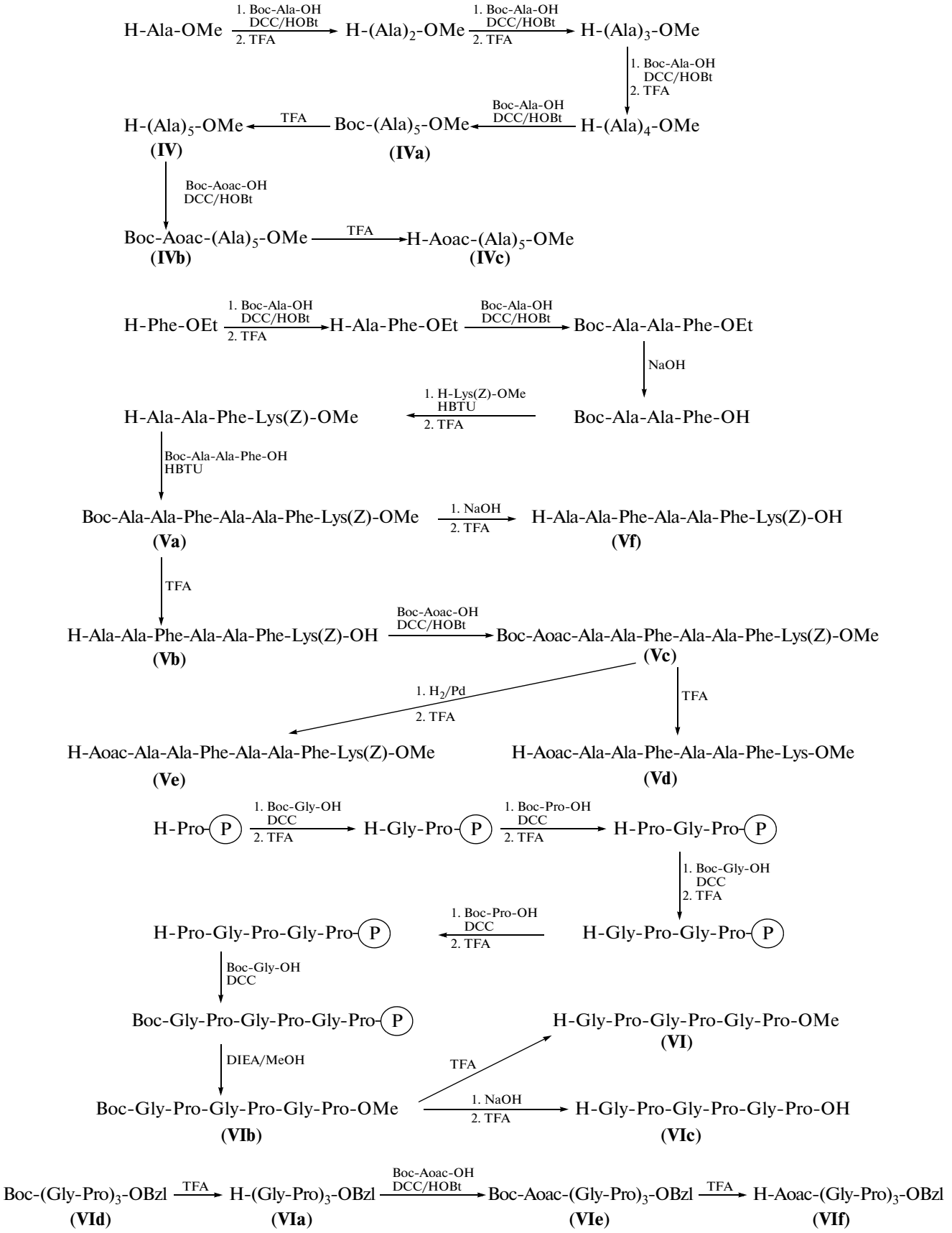
We chose two methods of the modification of the macrolide aldehyde group for the solution of the task. *The first method* is the attachment of a substituent through the $=NOCH_2CO-$ linker prepared by the interaction of the antibiotic aldehyde group with the amino group of 2-aminooxyacetic acid preliminarily attached to the α-amino group of an amino acid or peptide [13]. *The second method* implies the direct interaction of the macrolide aldehyde group with the amino acid α-amino group leading to a Schiff base followed by its reduction.

In published data, the synthesis of C20-amino-derivatives of Tyl-group antibiotics by the reductive amination of the macrolide aldehyde function with the use of various primary and secondary amines was described [19, 20, 22]. However, similar macrolide conjugates of amino acids and peptides have not yet been obtained. The modification by these two methods appears to allow for the preparation of constructs with different spatial characteristics and rigidity of the polypeptide chain near the PTC. For example, the substituted oximes on the basis of the peptide derivatives of the α-amino acid would have the characteristic conformational restrictions of the turn angle of the peptide chain due to the presence of a C=N double bond [23]. Oppositely, in the amino derivatives of macrolides available based on the second approach (reductive amination), an ordinary >C–N< bond would provide for the greater mobility of the peptide fragment.

We obtained the necessary derivatives of the antibiotics by the conjugation of Tyl, Des, and OMT with a set of amino acids and oligopeptides. First, we were interested in the amino acids whose side chains bear aromatic and heterocyclic radicals capable of both hydrophobic interactions and the formation of hydrogen bonds with the rRNA nucleic bases: phenylalanine, tyrosine, and 3-(uracil-1-yl)alanine.

Despite the aforementioned fact that the spatial structure of the growing polypeptide chain has not been established by direct methods, there are weighty arguments in published data that its short sites, prone to the formation of α-helices within proteins, are also in a helical conformation near the macrolide-binding site [24, 25]. This concept and the accounting for the conformational characteristics of single amino acid residues [26] determined our choice of model sequences (**IV**)–(**VI**) used in the synthesis of the Tyl and Des peptide derivatives. In particular, the H-Ala-Ala-Phe-Ala-Ala-Phe-Lys-OMe (**V**) sequence is characterized by a high inclination toward the formation of an α-helix, whereas neither ordered structure is possible for H-Gly-Pro-Gly-Pro-Gly-Pro-OX (**VI**) (X = Me) and (**VIa**) (X = Bzl).

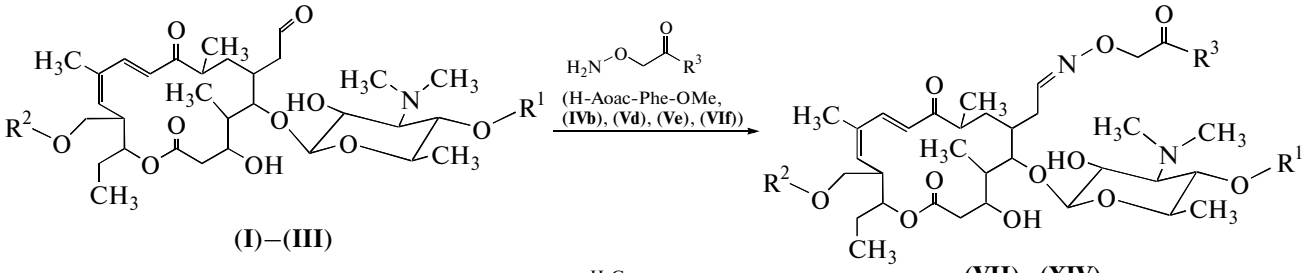
The synthesis of model peptides was carried out as follows (Scheme 1). The peptides Boc-Ala-Ala-Phe-OEt [14] and Boc-$(Ala)_5$-OMe (**IVa**) [27, 28] were obtained in a solution starting from H-Phe-OEt and H-Ala-OMe by a series of successive couplings with Boc-Ala-OH with the use of DCC in the presence of HOBt. The heptapeptide Boc-$(Gly\text{-}Pro)_3$-OMe (**VIb**) was synthesized by the solid-phase method; we described it in [14]. The peptide Boc-Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OMe (**Va**) was obtained by a block method using the scheme [3 + (3 + 1)] starting from H-Lys(Z)-OMe and Boc-Ala-Ala-Phe-OH with the use of HBTU for the coupling of fragments [29]. The inevitable partial racemization of the Phe residues at these stages was not studied specially; however, all of the side products, including diastereomers, were successfully removed by column chromatography, and the target heptapeptide was obtained in a chromatographically homogeneous state.


H-Ala-OMe
1. Boc-Ala-OH DCC/HOBt 2. TFA
H-(Ala)2-OMe
1. Boc-Ala-OH DCC/HOBt 2. TFA
H-(Ala)3-OMe
1. Boc-Ala-OH DCC/HOBt 2. TFA
H-(Ala)4-OMe
Boc-Ala-OH DCC/HOBt
Boc-(Ala)5-OMe
(IVa)
TFA
H-(Ala)5-OMe
(IV)
Boc-Aoac-OH DCC/HOBt
Boc-Aoac-(Ala)5-OMe
(IVb)
TFA
H-Aoac-(Ala)5-OMe
(IVc)
H-Phe-OEt
1. Boc-Ala-OH DCC/HOBt 2. TFA
H-Ala-Phe-OEt
Boc-Ala-OH DCC/HOBt
Boc-Ala-Ala-Phe-OEt
NaOH
Boc-Ala-Ala-Phe-OH
1. H-Lys(Z)-OMe HBTU 2. TFA
H-Ala-Ala-Phe-Lys(Z)-OMe
Boc-Ala-Ala-Phe-OH HBTU
Boc-Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OMe
(Va)
1. NaOH 2. TFA
H-Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OH
(Vf)
TFA
H-Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OH
(Vb)
Boc-Aoac-OH DCC/HOBt
Boc-Aoac-Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OMe
(Vc)
1. H2/Pd 2. TFA
TFA
H-Aoac-Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OMe
(Ve)
H-Aoac-Ala-Ala-Phe-Ala-Ala-Phe-Lys-OMe
(Vd)
H-Pro-(P)
1. Boc-Gly-OH DCC 2. TFA
H-Gly-Pro-(P)
1. Boc-Pro-OH DCC 2. TFA
H-Pro-Gly-Pro-(P)
1. Boc-Gly-OH DCC 2. TFA
H-Gly-Pro-Gly-Pro-(P)
1. Boc-Pro-OH DCC 2. TFA
H-Pro-Gly-Pro-Gly-Pro-(P)
Boc-Gly-OH DCC
Boc-Gly-Pro-Gly-Pro-Gly-Pro-(P)
H-Gly-Pro-Gly-Pro-Gly-Pro-OMe
(VI)
DIEA/MeOH
TFA
Boc-Gly-Pro-Gly-Pro-Gly-Pro-OMe
(VIb)
1. NaOH 2. TFA
H-Gly-Pro-Gly-Pro-Gly-Pro-OH
(VIc)
Boc-(Gly-Pro)3-OBzl
(VId)
TFA
H-(Gly-Pro)3-OBzl
(VIa)
Boc-Aoac-OH DCC/HOBt
Boc-Aoac-(Gly-Pro)3-OBzl
(VIe)
TFA
H-Aoac-(Gly-Pro)3-OBzl
(VIf)

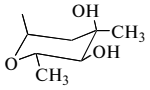
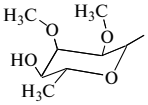
Scheme 1.

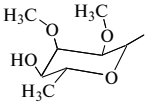
Compounds (**IV**), (**VIa**), and (**VIb**) were condensed with Boc-2-aminooxyacetic acid to obtain the peptide and amino acid derivatives of Tyl, Des, and OMT by the first method. The Z and Boc groups were then consecutively removed, and the peptides with a *N*-terminal aminooxy group that can selectively conjugate with aldehyde carbonyl were obtained.

The reaction of the formation of the macrolide oximes was carried out under the following conditions (Scheme 2): 1 equiv of Tyl (Des or OMT) and 1.5 equiv of aminooxyacetic acid or aminooxyacetylpeptide were incubated at 50°C for 12 h in a 0.4-M sodium acetate buffer (pH 4.7) or, in the case of the insufficient solubility of the peptide, in a mixture of this buffer and DMSO (1 : 1). The purification of the resulting derivatives of Tyl (**VII**)–(**IX**), Des (**X**)–(**XIII**), and OMT (**XIV**) was carried out by preparative TLC on silica gel in systems containing chloroform and methanol; the yields were 20–25% after the purification.

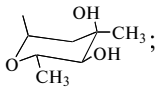
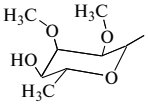


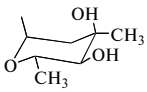
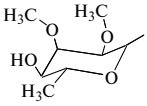
(**I**)–(**III**) (**VII**)–(**XIV**)

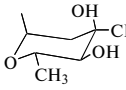
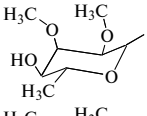
(**I**): R^1 =  ; R^2 = 

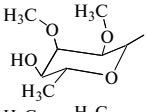
(**II**): R^1 = H; R^2 = 

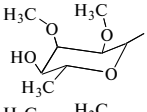
(**III**): R^1 = H; R^2 = H

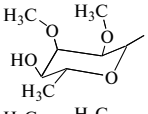
(**VII**): R^1 =  ; R^2 =  ; R^3 = OH

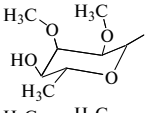
(**VIII**): R^1 =  ; R^2 =  ; R^3 = -Phe-OEt

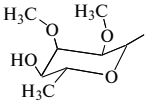
(**IX**): R^1 =  ; R^2 =  ; R^3 = -Ala-Ala-Ala-Ala-Ala-OMe

(**X**): R^1 = H; R^2 =  ; R^3 = -Phe-OEt

(**XI**): R^1 = H; R^2 =  ; R^3 = -Ala-Ala-Ala-Ala-Ala-OMe

(**XII**): R^1 = H; R^2 =  ; R^3 = -Ala-Ala-Phe-Ala-Ala-Phe-Lys-OMe

(**XIIa**): R^1 = H; R^2 =  ; R^3 = -Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OMe

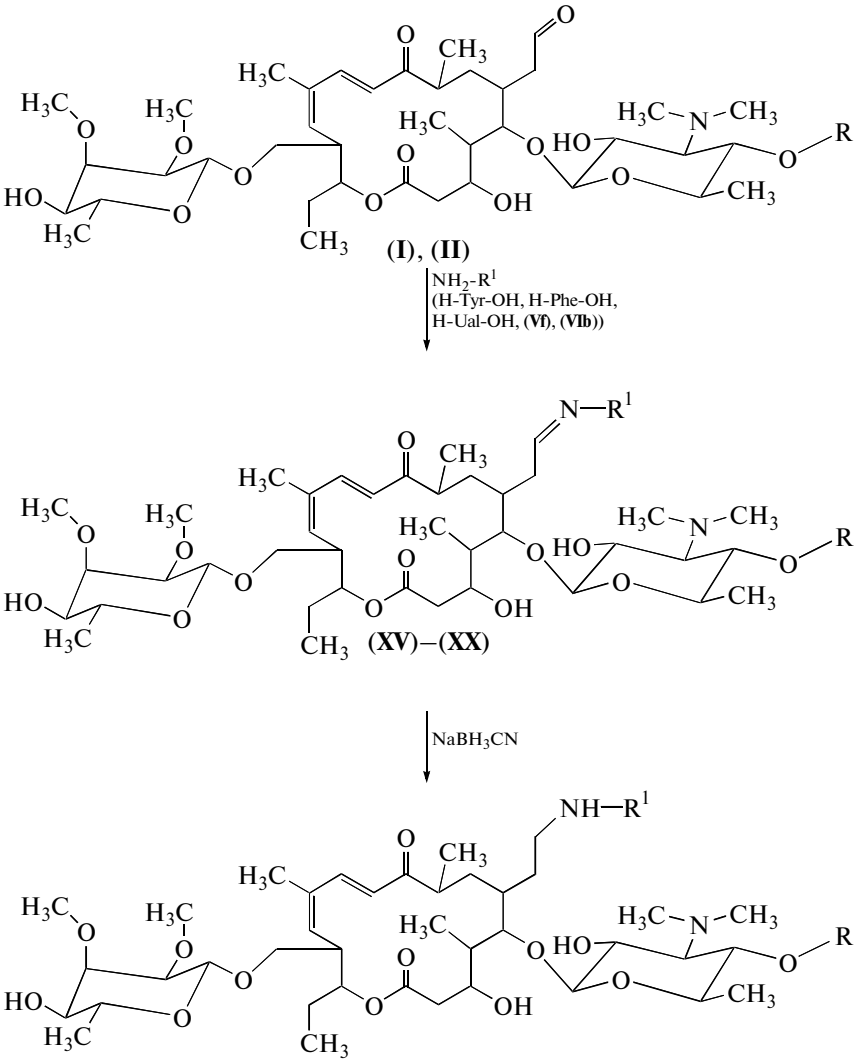
(**XIII**): R^1 = H; R^2 =  ; R^3 = -Gly-Pro-Gly-Pro-Gly-Pro-OBzl

(**XIV**): R^1 = H; R^2 = H; R^3 = -Phe-OEt

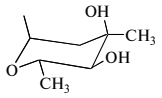
Scheme 2.

The homogeneity of the compounds isolated was assessed by TLC and HPLC, quantitative amino acid analysis showed the expected ratio of amino acid residues in products (**VIII**)–(**XIV**), and NMR and mass-spectrometric data were in complete agreement with the expected structures (Scheme 2).

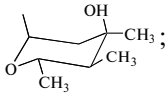
We applied a procedure similar to that described in [30] to obtain by the second method the Tyl and Des derivatives with aromatic amino acids (tyrosine and phenylalanine) and *DL*-3-(uracil-1-yl)alanine, a nucleoamino acid (Scheme 3). The reaction was carried out in absolute methanol with a twofold excess of amino acids in the presence of sodium methylate and 3-Å molecular sieves at room temperature. The resulting Schiff bases (**XV**)–(**XVIII**) were sufficiently stable in alcohol solutions and in neutral water solutions, which allowed for their isolation in a homogeneous state by preparative TLC on silica gel plates. The Des derivatives (conjugates (**XIX**) and (**XX**)) with peptides (**Vf**) and (**VIc**) were obtained by this method. However, the yields of these compounds were extremely low due to the formation of a great number of side products in the reaction mixtures, which was first the consequence of the hydrolysis of the lactone function by water split off during the formation of imine on the background of methylate.

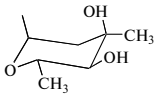


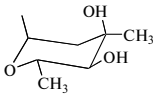
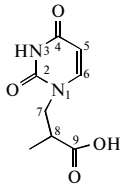
Scheme 3.

(**I**): R = 

(**II**): R = H

(**XV**): R =  ; R^1 = –CH(CH_2PhOH)COOH

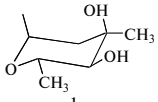
(**XVI**): R =  ; R^1 = –CH(CH_2Ph)COOH

(**XVII**): R =  ; R^1 = 

(**XVIII**): R = H; R^1 = –CH(CH_2PhOH)COOH

(**XIX**): R = H; R^1 = –CH(CH_3)CO-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OH

(**XX**): R = H; R^1 = –CH_2CO-Pro-Gly-Pro-Gly-Pro-OH

(**XXI**): R =  ; R^1 = –CH(CH_2PhOH)COOH

(**XXII**): R = H; R^1 = –CH_2CO-Pro-Gly-Pro-Gly-Pro-OH

Scheme 3. (Contd.).

The Schiff bases (**XV**) and (**XX**) were then reduced to the secondary amines (**XXI**) and (**XXII**) by sodium cyanoborohydride. In parallel, by the example of (**XX**), the reduction was carried out without the isolation of the corresponding imine by the direct addition of sodium cyanoborohydride to the mixture of components in which imine was generated in situ as described earlier [31].

We judged the substitution of the C20-macrolide aldehyde group by a comparison of the NMR spectra of the new compounds with the spectra of the starting Tyl and Des macrolides. The complete assignment of signals in the ^{1}H and ^{13}C NMR spectra of Tyl and Des was made based on two-dimensional NMR spectra COSY, HSQC, and HMBC. According to our data (see the Experimental section), the signal assignment in earlier work [32] has substantial inaccuracies. Earlier, we described the ^{1}H and ^{13}C NMR spectra of OMT [14].

We chose the derivatives (**VIII**), (**X**), and (**XIV**) obtained by the first method and (**XVII**) prepared by the second method for the comprehensive structural analysis. The proton spectra of the modified macrolides differed from the spectra of the starting compounds by the absence of a signal in the area of 9.6–9.7 ppm characteristic of an aldehyde proton and the appearance of a –CH=N proton in the area of 7.4–7.5 ppm. Moreover, the modification of, namely, the C20-aldehyde group was confirmed by the disappearance from the ^{13}C spectrum of the signal in the area of 202–204 ppm corresponding to a –CH=O carbon atom and the appearance of a signal at 150–160 ppm characteristic of the sp^2 carbon in Schiff bases and oximes. In this case, the signal at ~203 ppm corresponding to C9 carbon was retained. Two sets of signals corresponding to two (*Z* and *E*) isomers with a different orientation of the substituent are often observed for oximes and Schiff bases related to Tyl in NMR spectra [33]. Note that in the case of the solution of our compounds in $CDCl_3$, only one set of signals is observed, which appears to be explained by the greater conformational preferability of one of the isomers (*anti*-isomer).

A preliminary study of the biological properties of the series of new derivatives of the macrolide antibiotics, the synthesis of which is described in this work, showed that many of them is specifically bound to the ribosomal RT of *E. coli* and inhibit protein synthesis in the cell-free system of transcription–translation [34, 35] with an efficiency comparable and, in some cases, exceeding that of the starting macrolides. These data imply that the more comprehensive structural and functional analysis of the series of amino acid and peptide derivatives of macrolides we are carrying out now could be a source of valuable information regarding the peculiarities of the interaction of the growing polypeptide chain with the RT.

EXPERIMENTAL

In this work, we used amino acids and reagents from Fluka and Bachem (Switzerland), Merck (Germany), and Reanal (Hungary). The peptide Boc-(Gly-Pro)$_3$-OBzl (**VId**) was a kind gift of Academician N.F. Myasoedov and his coworkers (Institute of Molecular Genetics, Russian Academy of Sciences).

TLC was carried out on Kieselgel 60F_{254} plates (Merck, Germany) with the use of the following solvent systems: (A) 9 : 1 chloroform–methanol, (B) 100 : 50 : 2 benzene–acetone–acetic acid; (C) 4 : 1 chloroform–methanol; (D) 85 : 10 : 5 chloroform–ethanol–acetic acid; (E) 14 : 1 : 5 isopropanol-25% ammonia–water; (F) 65 : 4 : 25 chloroform–water–methanol; (G) 15 : 1 chloroform–methanol; (H) 7 : 3 chloroform–methanol; (I) 6 : 1 chloroform–methanol; (J) 10 : 1 chloroform–methanol; (K) 8 : 1 : 0.1 dichloromethane–methanol–25% ammonia; (L) 65 : 4 : 25 chloroform–25% ammonia–methanol; and (M) 1 : 2 chloroform–methanol. The compounds absorbing UV light were detected by a Brumberg chemiscope, and the amino acids and peptides containing free amino groups were detected with a standard ninhydrin reagent. The Boc-protected amino derivatives were similarly detected after the keeping of the chromatogram in vapors of trifluoroacetic acid. Des, Tyl, and their derivatives were detected with a 0.4% solution of 2,4-dinitrophenylhydrazine in 2 M HCl.

UV absorption spectra were registered on a Cary 50 Bio spectrophotometer (Varian, United States).

Amino acid analysis was carried out on a Hitachi 835 analyzer (Japan).

The reversed-phase HPLC was performed on a Milichrom A-02 (EKONOVA, Russia) on a Pronto-SIL-120-5-C18 AQ column (2.0 × 75 mm, 5 μm) (Macherey-Nagel, Germany) in an acetonitrile gradient in 0.1% trifluoroacetic acid; the elution rate was 100 μl/min and the detection was at 214 and 280 nm.

Silica gel 60 (0.063–0.2 mm, Fluka) was used for column chromatography.

The mass-spectrometric analyses with the use of matrix-assisted ionization (MALDI–TOF) were carried out on Ultraflex and Autoflex mass spectrometers from Bruker Daltonik (Germany).

The values of the optical rotation of substances were measured on a VNIEKIPRODMASH EPO 1A instrument at a wavelength of 589 nm.

The ^{1}H and ^{13}C NMR spectra of the compounds under study were registered on Bruker AM-300, Bruker DRX-500, and Bruker AV-600 spectrometers with working frequencies of 300, 500, and 600 MHz, respectively, for the ^{1}H nuclei. The multiplicities of signals in the ^{13}C spectra were determined with the use of INEPT experiments. The two-dimensional NMR COSY spectra were registered in a magnitude representation; the transformation of the two-dimensional HSQC and HMBC spectra was carried out in a phase-sensitive regime. When registering the HSQC spectra a J filter was used, optimal for the constants ^{13}C-H through one bond (of the order of 135 Hz). In the case of HMBC, a transmitting J filter was used for long-range constants ^{13}C-H (of a 7-Hz order) and the refocusing of the ^{13}C-H constants through one bond. The registration of the spectra was carried out in a $CDCl_3$ solution at 303 K. The chemical shifts are given in ppm, and spin–spin coupling constants are given in Hz. The data of ^{13}C NMR and two-dimensional experiments COSY, HSQC, and HMBC were used for signal assignment.

Starting antibiotics. In this work, we used tylosin (**I**) produced by ZAO Mosagrogen (Russia); desmycosin (**II**) and OMT (**III**) were obtained by the acidic hydrolysis of tylosin (**I**) [13, 14].

Tylosin (I): ^{1}H NMR (600 MHz): 0.93 (3 H, t, *J* 7.3, H17), 0.99 (3 H, d, *J* 6.9 H18), 1.21 (3 H, d, J6.8, H5'CH_3), 1.22 (3 H, s, H3"CH_3), 1.25 (3 H, d, H21), 1.26 (3 H, d, *J* 6.3, H5'CH_3), 1.28 (3 H, d, *J* 6.2, H5'"CH_3), 1.48 (1 H, m, H7), 1.62 (3 H, m, H4, H7, H16), 1.76 (1 H, dd, *J* 3.9 and 3.8, H2"), 1.78 (3 H, s, H22), 1.88 (1 H, m, H16), 1.93 (1 H, d, *J* 16.4, H2), 2.03 (1 H, d, *J* 13.6, H2"), 2.16 (2 H, m, H6, H19), 2.48 (6 H, 2 H, s, m, $N(CH_3)_2$, H2, H3'), 2.60 (1 H, m, H8), 2.87 (1 H, m, H19), 2.95 (1 H, m, H14), 3.02 (1 H, dd, H2'"), 3.17 (1 H, m, H4'"), 3.26 (2 H, m, H4', H5'), 3.48 (3 H, s, H2'" OCH_3), 3.52 (3 H, m, H2', H23, H5'"), 3.60 (3 H, s, H3'" OCH_3), 3.70 (1 H, d, *J* 9.0, H5), 3.74 (1 H, t, *J* 3.03, H3'"), 3.82 (1 H, d, *J* 10.4, H3), 3.91 (1 H, dd, H23), 4.04 (1 H, m, H4"), 4.21 (1 H, d, *J* 7.4, H1'), 4.55 (1 H, d, *J* 7.8, H1'"), 4.90 (1 H, m, H15), 5.06 (1 H, d, *J* 3.4, H1"), 5.90 (1 H, d, *J* 10.5, H13), 6.30 (1 H, d, *J* 15.5, H10), 7.30 (1 H, d, *J* 15.3, H11), 9.67 (1 H, m, H20).

^{13}C NMR (150.92 MHz): 9.12 (C18), 9.66 (C17), 12.98 (C22), 17.36 (C21), 17.75 (C5'" CH_3), 18.24 (C5' CH_3), 19.02 (C5" CH_3), 25.47 (C16), 30.00 (C6), 32.50 (C7), 39.36 (C2), 40.16 (C4), 40.90 (C3' $N(CH_3)_2$), 40.91 (C2"), 41.96 (C3" CH_3), 43.75 (C14), 44.70 (C8), 45.06 (C19), 59.71 (C2'" OCH_3), 61.75 (C3'" OCH_3), 68.76 (C3'), 69.06 (C3, C23, C4"), 70.63 (C5'"), 72.67 (C2', C4'"), 73.19 (C5"), 75.18 (C15, C4', C5'), 76.41 (C3"), 79.81 (C3'"), 81.93 (C5), 81.94 (C2'"), 96.50 (C1"), 101.08 (C1'"), 103.96 (C1'), 119.31 (C10), 134.88 (C12), 142.31 (C13), 148.07 (C11), 173.93 (C1), 202.47 (C20), 203.03 (C9).

Desmycosin (II): ^{1}H NMR (600 MHz): 0.98 (3 H, t, *J* 7.4 H17), 1.04 (3 H, d, *J* 6.8, H18), 1.22 (3 H, d, H21), 1.28 (6 H, m, H5'CH_3, H5'"CH_3), 1.32 (1 H, d, *J* 5.4, H3"CH_3), 1.50 (1 H, m, H7), 1.63 (3 H, m, H4, H7, H16), 1.80 (3 H, s, H22), 1.88 (1 H, m, H16), 1.95 (1 H, d, *J* 16.5, H2), 2.11 (1 H, m, H6), 2.39 (3 H, m, H2, H3', H19), 2.50 (6 H, m, $N(CH_3)_2$), 2.61 (1 H, m, H8), 2.95 (2 H, m, H14, H19), 3.05 (1 H, m, H2'", H4'), 3.19 (1 H, m, H4'"), 3.28 (1 H, m, H5'), 3.50 (3 H, s, H2'"OCH_3), 3.55 (3 H, m, H2', H23, H5'"), 3.63 (3 H, s, H3'"OCH_3), 3.75 (2 H, m, H3'", H5), 3.85 (1 H, d, *J* 10.6, H3), 4.00 (1 H, dd, H23),

4.26 (1 H, d, *J* 7.3, H1'), 4.57 (1 H, d, *J* 7.8, H1'''), 4.90 (1 H, m, H15), 5.95 (1 H, d, *J* 10.3, H13), 6.27 (1 H, d, *J* 15.3, H10), 7.33 (1 H, d, *J* 15.0, H11), 9.70 (1 H, s, H20).

^{13}C NMR (150.92 MHz): 8.97 (C18), 9.66 (C17), 12.97 (C22), 15.30 (C21), 15.70 (C5''' CH_3), 15.70 (C5' CH_3), 25.45 (C16), 29.65 (C6), 31.54 (C7), 39.37 (C2), 40.16 (C4), 41.67 (C3'N$(CH_3)_2$), 43.76 (C14), 44.66 (C8), 45.05 (C19), 59.72 (C2'''OCH_3), 61.79 (C3'''-OCH_3), 69.05 (C23), 70.11 (C3'), 70.54 (C5'''), 70.77 (C2'), 70.99 (C3), 72.64 (C4'''), 73.34 (C5'), 73.43 (C4'), 75.09 (C15), 79.83 (C3'''), 81.84 (C5), 81.89 (C2'''),101.06 (C1'''), 103.96 (C1'),118.52 (C10), 134.86 (C12), 142.24 (C13), 148.00 (C11), 173.86 (C1), 202.90 (C20), 203.09 (C9).

Synthesis of Peptides

Boc-$(Ala)_5$-OMe (IVa). *Boc-$(Ala)_n$-OMe* were obtained by coupling Boc-Ala-OH with H-$(Ala)_{n-1}$-OMe (n = 2–5) using DCC in the presence of HOBt by the procedure described for Boc-$(Ala)_2$-OMe in [14]. In this manner, we obtained:

Boc-$(Ala)_3$-OMe (65%), mp 182–184°C (from isopropyl ether, dec.); $[\alpha]_D^{20}$ –20° (*c* 1, DMF), R_f 0.70 (A) and 0.45 (B) (published data: mp 185°C [36] and 193–194°C [27]; $[\alpha]_D^{20}$ –48° (*c* 1, CH_2Cl_2) [36], $[\alpha]_D^{20}$ –8.48° (1,1,1,3,3,3-hexafluoropropan-2-ol)[27]);

Boc-$(Ala)_4$-OMe (48%), mp 148–150°C (from ethyl acetate, dec.); $[\alpha]_D^{20}$ –35° (*c* 1, DMF), R_f 0.62 (A), R_f 0.19 (B) (published data: mp 246°C [37], $[\alpha]_D^{20}$ –39.5° (*c* 1, DMF) [36], $[\alpha]_D^{20}$ –8.4° (1,1,1,3,3,3-hexafluoropropan-2-ol)[27]);

Boc-$(Ala)_5$-OMe (IVa), (28%), mp 266–268°C (from ethyl acetate, dec.); $[\alpha]_D^{20}$ –45° (*c* 1, DMF), τ 15 min (gradient 0–60% acetonitrile in 0.1% TFA for 20 min); R_f 0.10 (A), 0 (B) (published data: $[\alpha]_D^{20}$ –8.31° (1,1,1,3,3,3-hexafluoropropan-2-ol)[27]).

Boc-Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OMe (Va). *Boc-Ala-Ala-Phe-Lys(Z)-OMe.* HBTU (1 equiv) and, after 5 min, H-Lys(Z)-OMe (1.1 equiv) were added to a solution of Boc-Ala-Ala-Phe-OH [14] in DMF under cooling (0°C) and stirring. The reaction mixture was kept for 2 h, ten times diluted with water, and three-times extracted with chloroform. The extract was three-times washed with 5% Na_2CO_3, water (one time), 0.05 M H_2SO_4 (three times), a saturated solution of NaCl (one time), dried by $MgSO_4$, and evaporated on a rotary evaporator. The residue was recrystallized from ethyl acetate. Yield 86% of white crystals, mp 165–169°C; R_f 0.40 (B), 0.85 (C), 0.90 (D); amino acid analysis: Ala 1.68 (2), Phe 1.00 (1), Lys 1.05 (1).

TFA·H-Ala-Ala-Phe-Lys(Z)-OMe. TFA (20 equiv) were added to Boc-Ala-Ala-Phe-Lys(Z)-OMe (1 equiv), the solution was stirred for 1 h at room temperature, TFA was removed by evaporation on a rotary evaporator, and the residue was dried in a vacuum desiccator over NaOH. A white crystalline substance was obtained; yield 99%; mp 158–159°C; R_f 0.85 (E), 050 (F).

Boc-Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OMe (Va). HBTU (1 equiv) were added under cooling (0°C) and stirring to a solution of Boc-Ala-Ala-Phe-OH (1 equiv) in DMF. The mixture was stirred for 5 min and then treated with TFA·H-Ala-Ala-Phe-Lys(Z)-OMe (1.1 equiv) dissolved in DMF and neutralized with 1.1 equiv of *N,N*-diisopropylethylamine. The mixture was stirred for 5.5 h at 0°C, ten-times diluted with water, and four-times extracted with chloroform. The extract was washed with 5% Na_2CO_3 (three times), water (once), 0.05 M H_2SO_4 (three times), and saturated solution of NaCl (once). Chloroform was removed in a vacuum, and the residue was precipitated with 10 volumes of petroleum ether from 1 volume of ethyl acetate. The precipitated solid (75%) was then purified by column chromatography on silica gel using the chromatographic system G as an eluent. Compound (**Va**) resulted; yield 41%; τ (HPLC) 19 min (gradient from 15 to 80% for 20 min); MS, *m/z* (found/calc.): 973.5/973.12, 873.4/873.0 (*M* – Boc); 839.4/839.0 (*M* – Z); R_f 0.71 (A), 0.66 (D), 0.52 (G). Amino acid analysis: Ala 3.78 (4), Phe 1.68 (2), Lys 1.00 (1).

Preparation of *N*-aminooxyacetylpeptides. Peptides (**IVa**), (**Va**), and **VIb**) were treated with TFA (20 equiv) for 1 h at room temperature. TFA was removed in a vacuum, and a solution of *N,N*-diisopropylethylamine (1 equiv) in DMF was added to the residue.

HOBT (1.3 equiv) and DCC (1.3 equiv) were added to a solution of Boc-2-aminooxyacetic acid (Boc-Aoac-OH, 1 equiv) in DMF under cooling (0°C) and stirring. After 1 h, phenylalanine ethyl ester or a peptide (**IV**), (**Vb**), or (**VIa**) (1 equiv) in DMF was added to the solution. The mixture was stirred for 4 h at room temperature, ten-times diluted with water, and extracted with ethyl acetate. The extract was washed with 0.1 M HCl (three times), 5% Na_2CO_3 (three times) and water, dried with $MgSO_4$, and evaporated. We obtained:

Boc-Aoac-Phe-OEt, yield 77%, τ (HPLC) 8 min (gradient 0–60% for 20 min), R_f 0.87 (A), 0.75 (B), 0.92 (C), 0.95 (D);

Boc-Aoac-$(Ala)_5$-OMe (IVb), yield 60%; R_f 0.75 (D);

Boc-Aoac-Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OMe (Vc), yield 73%, R_f 0.66 (A), 0.57 (D);

Boc-Aoac-$(Gly\text{-}Pro)_3$-OBzl (VIe), yield 35%, R_f 0.50 (A), 0.65 (C).

Boc-Aoac-Ala-Ala-Phe-Ala-Ala-Phe-Lys-OMe. 10% Pd/C (50% of the mass of the starting peptide) and 4 equiv of ammonium formate were added to a solution of Boc-Aoac-Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OMe (1 equiv) in DMF, and the mixture was stirred at room temperature for 24 h. The catalyst was

filtered off, the filtrate was evaporated in a vacuum, and the residue was distributed between butanol and a saturated solution of NaCl (5 : 1). Butanol was removed on a rotary evaporator, and the residue was dried in a vacuum over $CaCl_2$; yield 85%; R_f 0.42 (B), 0.71 (D).

Preparation of the substituted oximes of macrolides. Boc-aminooxyacetylpeptides were treated with TFA (20 equiv) for 1 h at room temperature, and TFA was removed in a vacuum.

A solution of Tyl, Des, or OMT (1 equiv) in a 0.4-M sodium acetate buffer (pH 4.7) was mixed with *N*-aminooxyacetylpeptide (**IVc**), (**Vd**), (**Ve**), or (**VIf**) in an equal volume of DMSO or 2-aminooxyacetic acid in an equal volume of a 0.4-M sodium acetate buffer (pH 4.7). The reaction mixture was incubated at 50°C for 12 h, diluted with water, adjusted to pH 9, and extracted with chloroform. The target compound was isolated by preparative TLC in a chromatographic system A, C, or H, eluting from silica gel with methanol.

There were obtained in this manner:

Tyl-Aoac-OH (VII), yield 28%, τ (HPLC) 12.5 min (gradient 20–80% of acetonitrile in 0.1% TFA for 25 min); *m/z* (found/calc.): 988.7/988.5; R_f 0.77 (C), 0.73 (H).

Tyl-Aoac-Phe-OEt (VIII), yield 21%; τ (HPLC) 19.5 min (gradient 0–60% of acetonitrile in 0.1% TFA for 25 min; *m/z* (found/calc.): 1164.7/1164.4; R_f 0.45 (A), 0.67 (C); The ^{1}H NMR, COSY, and HSQC spectra (600 MHz, 303 K, $CDCl_3$) differ from the spectra of tylosin by the absence of a signal of the aldehyde proton (9.67 ppm), the appearance of oxime proton H20 (br. s at 7.42 ppm), and the signals of the Aoac-Phe-OEt residue: 1.20 (3H, t, $OCH_2\underline{CH_3^{Phe}}$, 3.15 (3 H, m, CH_2^{Phe}, C4'''), 4.17 (2H, m, $O\underline{CH_2}CH_3^{Phe}$), 4.52 (1 H, dd, CH_2^{Aoac}), 4.88 (1 H, m, $\underline{CH}CH_2^{Phe}$), 6.81 (1 H, br s, NH^{Phe}), 7.27 (6 H, m, Phe, H11), 7.42 (1 H, t, *J* 5.75, H20).

Tyl-Aoac-(Ala)$_5$-OMe (IX), yield 20%; τ (HPLC) 18.5 min (gradient 0–60% for 25 min); *m/z* (found/calc.): 1358.2/1358.4; R_f 0.76 (H).

Des-Aoac-Phe-OEt (X); yield 20%; RT (HPLC) 14.1 min (gradient 20–80% for 25 min; *m/z* (found/calc.): 1020.7/1020.2; R_f 0.79 (A), 0.76 (J), 0.80 (K); The ^{1}H NMR, COSY, HSQC (600 MHz, 303 K, $CDCl_3$) differ from the desmycosin spectra by the absence of the signal of the aldehyde proton (9.70 ppm) and the appearance of an oxime proton H20 (br s at 7.45 ppm) and signals of the residue Aoac-Phe-OEt: 1.20 (3 H, t, $OCH_2\underline{CH_3^{Phe}}$), 3.16 (5 H, m, CH_2^{Phe}, C4''', C4'), 4.17 (2 H, m, $O\underline{CH_2}CH_3^{Phe}$), 4.52 (1 H, dd, CH_2^{Aoac}), 4.90 (1 H, m, $\underline{CH}CH_2^{Phe}$), 6.85 (1 H, br s, NH^{Phe}), 7.29 (5 H, m, Phe), 7.45 (1 H, br s, H20).

Des-Aoac-(Ala)$_5$-OMe (XI); yield 18%; τ (HPLC) 16.8 min (gradient 0–60% for 25 min); *m/z* (found/calc.): 1214.0/1214.3; R_f 0.50 (H).

Des-Aoac-Ala-Ala-Phe-Ala-Ala-Phe-Lys (Z)-OMe (XIIa); yield 21%; *m/z* (found/calc.): 1698.9/1699.9 (*M* + H), 1685.0/1685.8 (*M* − CH_3); R_f 0.64 (C), 0.89 (H), 0.40 (I).

Des-Aoac-Ala-Ala-Phe-Ala-Ala-Phe-Lys-OMe (XII); yield 23%; *m/z* (found/calc.): 1551.2/1550.7 (*M* − CH_3); R_f 0.10 (H).

Des-Aoac-Gly-Pro-Gly-Pro-Gly-Pro-OBzl (XIII); yield 26%; τ (HPLC) 18.5 min (gradient 0–60% for 25 min); *m/z* (found/calc.): 1397.6/1397.5; R_f 0.80 (H).

OMT-Aoac-Phe-OEt (XIV); yield 23%; τ (HPLC) 13.9 min (gradient 20–80% for 25 min); *m/z* (found/calc.): 846.5/846.0; R_f 0.83 (A), 0.98 (H), 0.82 (J), 0.8/2; The ^{1}H NMR differs from the OMT spectrum by the absence of an aldehyde proton (at 9.64 ppm), the appearance of an oxime proton H20 signal (br s at 7.40 ppm), and the presence of signals of the Aoac-Phe-OEt residue.

Preparation of substituted imines of tylosin and desmycosin. A 3.5-M solution of sodium methylate in absolute methanol (4 equiv) and 3-Å molecular sieves were added to an amino acid or a peptide (**Vf**) or (**VIc**) (2 equiv) to solution of 1 equiv tylosin (desmycosin) in absolute methanol. The mixture was stirred for 12 h at room temperature, the solvent was removed in a vacuum, and the residue was separated on a glass plate coated with a silica gel layer in system H, L, or M. The resulting compound was eluted from silica gel by methanol.

Tyl=Tyr-OH[3] (XV); yield 25%; *m/z* (found/calc.): 1079.7/1079.3; R_f 0.25 (C), 0.38 (H); λ_{max} (UV) 285 nm ($\varepsilon_{285} = 1.9 \times 10^4$ M$^-$ cm^{-1}), 340–345 nm (shoulder), 385 nm ($\varepsilon_{385} = 1.3 \times 10^3$ M^{-1}cm^{-1}); ^{1}H NMR (600 MHz, 303 K, $CDCl_3$) differs from the spectrum of tylosin by the absence of an aldehyde proton signal (at 9.70 ppm) and the appearance of the proton H20 of the Schiff base (7.45 ppm), and also the presence of the signals of tyrosine residue: 2.16 (2 H, m, H6, H19), 2.87 (1 H, m, H19), 3.78 (1 H, m, CH_2^{Tyr}), 4.74 (1 H, m, $CH^{\alpha\ Tyr}$), 7.37 (5 H, m, H11, Tyr), 7.45 (1 H, br m, H20).

Tyl=Phe-OH (XVI); yield 16%; *m/z* (found/calc.): 1063.7/1063.1; R_f 0.30 (C), 0.63 (H); λ_{max} (UV) 285, 385 nm.

Tyl=Ual-OH (XVII) yield 21%; *m/z* (found/calc.) 1097.7/1097.1; R_f 0.67 (E), 0.69 (L); λ_{max} (UV) 275 nm ($\varepsilon_{275} = 6.1 \times 10^4$ M^{-1}cm^{-1}; ^{1}H NMR (600 MHz, 303 K, $CDCl_3$): differs from the tylosin spectrum by the absence of an aldehyde proton signal (9.70 ppm) and the presence of an H20 signal of the Schiff base (br m at 7.45 ppm) and the signals of Ual residue: 3.75 (1 H,

[3] A double hyphen between the abbreviated name of the antibiotic and amino acid or peptide substituent designates the binding by the Schiff base formation.

m, H8 of C**H**^{Ual}), 4.35 (7 H, m, H15, H1''', H1', H7 C$\mathbf{H}_2^{Ual}$), 5.63 (1 H, m, H5^{Ual}), 7.35 (2 H, H11, H6^{Ual}), 7.45 (1 H, br s, H20).

Des=Tyr-OH (XVIII); yield 18%; *m/z* (found/calc.): 933.6/935.0; R_f 0.15 (F), 0.30 (M).

Des=Ala-Ala-Phe-Ala-Ala-Phe-Lys(Z)-OH (XIX), *m/z* (found/calc.): 1477.2/1477.6 (M – Z); R_f 0.15 (M).

Des=Gly-Pro-Gly-Pro-Gly-Pro-OH (XX); *m/z* (found/calc.): 1231.0/1234.3; R_f 0.20 (M).

Preparation of C20-aminoderivatives of tylosin and desmycosin. Sodium cyanoborohydride (4 equiv) was added to imminoderivative (**XV**) or (**XX**) (1 equiv) in methanol. The mixture was stirred for 12 h, and the solvent was removed in a vacuum. The residue was dissolved in water, adjusted in the cold to pH 3 with 0.1 N HCl and then to pH 7 with saturated sodium bicarbonate and extracted with chloroform. The combined chloroform extracts were washed with water, dried with sodium sulfate, evaporated in a vacuum, and the residue was subjected to chromatographic separation on a silica gel plate in system C for (**XXI**) or M for (**XXII**).

Tyl-Tyr-OH (XXI); yield 23%; *m/z* (found/calc.): 1081.9/1081.0; R_f 0.56 (C), 0.68 (H); λ_{max} (UV) 225, 285 nm; ^{1}H NMR (600 MHz, 303 K, $CDCl_3$): unlike the spectrum of Tyl=Tyr, the signals at 7.45 and 4.74 characteristic of Schiff base are absent, the signals of the reduced Schiff base appear, signals H19 of tylosin are shifted to a stronger field (2.16–2.87 ppm in a non-reduced Schiff base; 1.5–1.7 ppm in a reduced Schiff base): 1.5–1.7 (1 H, m, H19), 3.51 (1 H, m, H20), 3.63 (1 H, m, CH$^{\alpha\,Tyr}$), 3.74 (1 H, s, NH^{Tyr}), 3.76 (1 H, m, CH_2^{Tyr}), 7.37 (5 H, m, H11, Tyr).

Des-Gly-Pro-Gly-Pro-Gly-Pro-OH (XXII); *m/z* (found/calc.): 1233.0/1235.3; R_f 0.38 (M).

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