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ANTIBACTERIAL GLYCOPEPTIDE ANTIBIOTICS (REVIEW)

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The structures of the most important antibacterial glycopeptide antibiotics and the mechanisms of their activity and resistance to them are examined. Researches on the total synthesis of antibiotics and model compounds are discussed. The chemical modification of antibiotics (changing the amino acid composition) and also the modification of fragments of the molecule that do not take part in interaction with the target but make it possible to overcome the resistance of bacteria to antibiotics of this group are discussed.

1. THE STRUCTURE OF GLYCOPEPTIDE ANTIBIOTICS, THE MECHANISM OF THEIR ACTIVITY, AND THE NATURE OF THE RESISTANCE OF BACTERIA TO THEM

The glycopeptide antibiotics vancomycin and teikoplanin are the main medicinal agents in the fight against infections due to Gram-positive bacteria with multiple drug resistance [1, 2]. They are used for the treatment of various types of sepsis, endocarditis, colitis, pneumonia, osteomyelitis, and pulmonary abscesses.

Glycopeptide antibiotics have a complex structure, based on a heptapeptide skeleton, where all the radicals of the aromatic amino acids are linked together, forming three or four additional macroheterocycles M(2-O-4), M(4-O-6), M(5-7) and for certain groups of antibiotics M(1-O-3). Below we give the complete structures of the most important natural glycopeptides: Ristomycin A (ristocetin A); vancomycin; teikoplanin; eremomycin; antibiotic LY264826 (A82846B, chloroeremomycin); orienticin C (dechloroeremomycin); antibiotic A-40926. For teikoplanin and antibiotic A-40926, which represent a set of structurally similar substances, the structural formulas of only the principal components are given. The amino acid residues 1 and 3 of vancomycin and eremomycin are aliphatic in nature and are not linked to each other (a vancomycin type of aglycone), whereas the corresponding fragments in teikoplanin and ristomycin are residues of aromatic amino acids, which are linked to each other forming a fourth macrocycle M(1-O-3) (a ristomycin type of aglycone).

The glycopeptide antibiotics were called dalbaheptides—"DALBAHEPTIDES" from DAL (D-Ala-D-Ala), B (binding), A (antibiotics), and HEPTAPEPTIDic structure, i.e., D-alanyl-D-alanine-binding heptapeptide antibiotics [3]. The name underlines the uniqueness of the mechanism of the action of these antibiotics, which involves suppression of the synthesis of the peptidoglycan in the cell wall of the bacteria as a result of strong bonding with the disaccharidepentapeptide fragment, which has the C-terminal sequence -Lys-D-Ala-D-Ala [4]. A major role in the formation of the complex is played by the peptide core of the antibiotic (Fig. 1), which forms five hydrogen bonds with the ligand. Here, the side radicals of the amino acids form the walls of a "binding pocket." By forming a stable complex with the substrate of the enzymatic reactions the antibiotic prevents subsequent biochemical processes and consequently leads to the death of the bacteria.

The method for the construction of the peptidoglycan in the cell wall of the various bacteria is universal and conservative. Even Gram-negative bacteria resistant to glycopeptides have a very similar method of construction of the cell wall using the -D-Ala-D-Ala fragment. Their resistance is explained by the presence of an outer lipopolysaccharide layer, which blocks access to the peptidoglycan in the bulky molecules of the glycopeptide antibiotics. It is in the effect on the substrate and not on the enzyme that we see the main reason for the comparatively slow development of the resistance of microorganisms to the glycopeptides, since its development requires multiple mutations.

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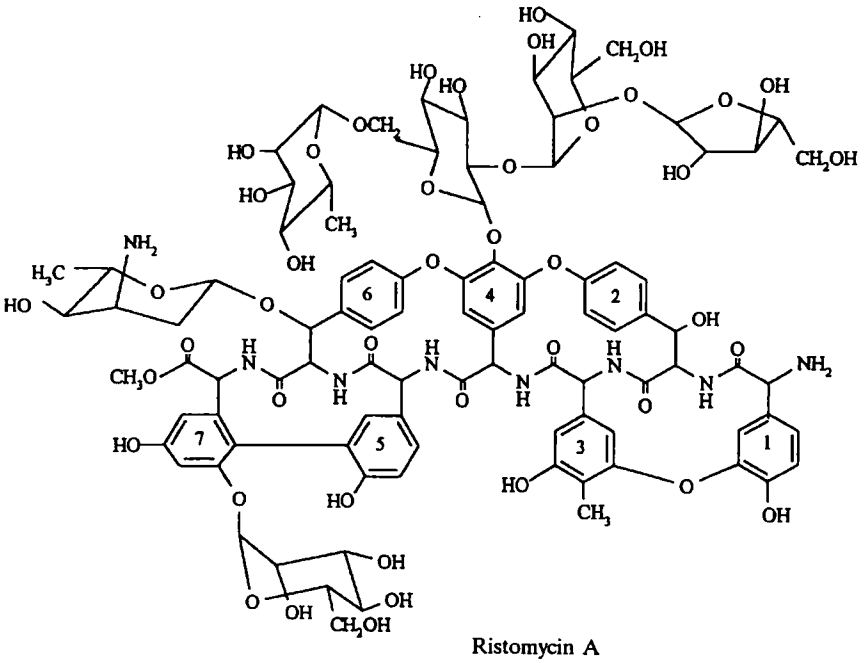
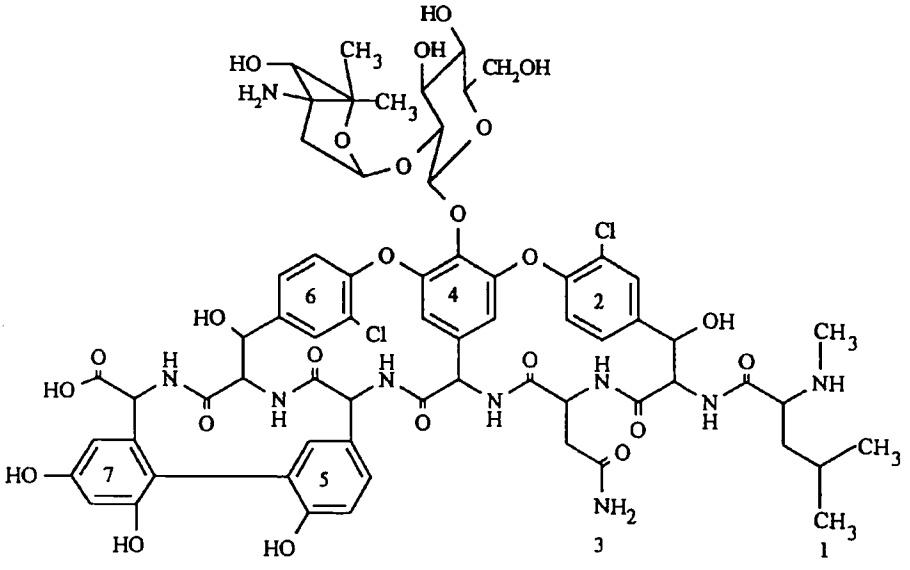
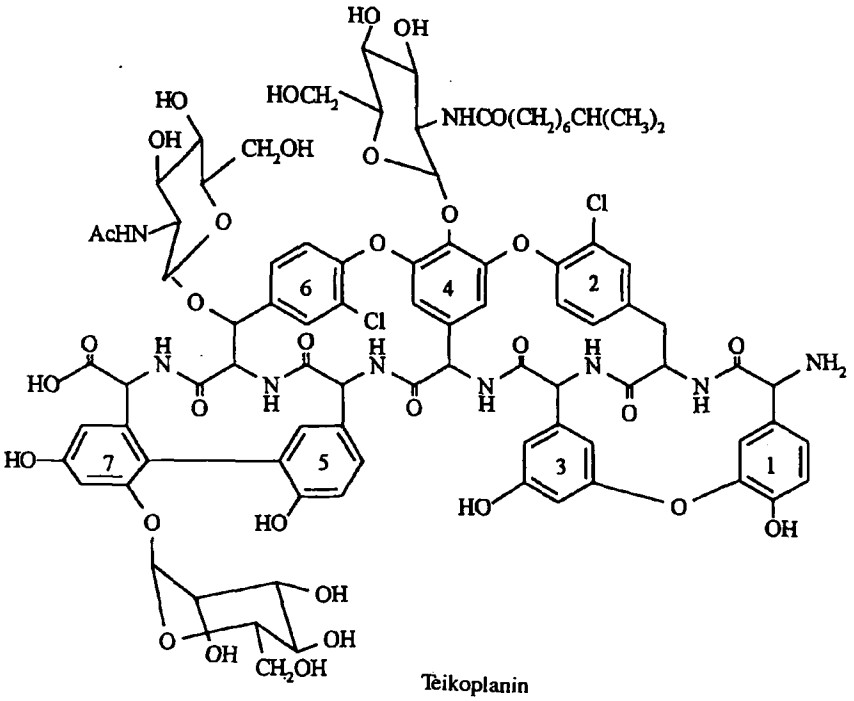


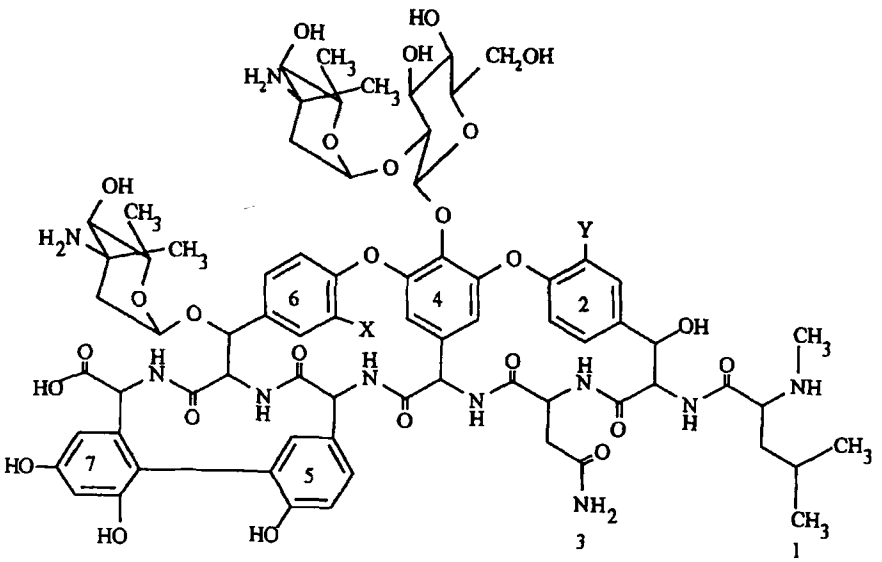
Ristomycin A



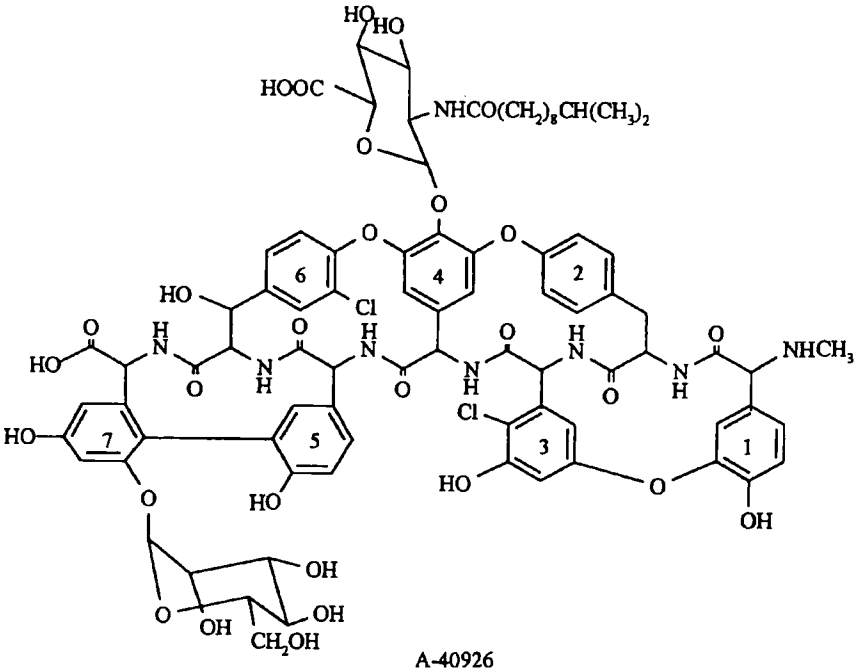
Vancomycin



Teikoplanin



Eremomycin	X= H, Y= Cl
LY264826	X= Y= Cl
Orienticin C	X= Y= H



A-40926

The development of resistance to these antibiotics was not observed for a long time. However, the more and more frequent use of vancomycin and teikoplanin in clinical practice and also the use of an antibiotic of this group avoparcin in agriculture has led to the appearance of multiresistant strains of enterococcus, resistant also to glycopeptide antibiotics [5]. The widespread occurrence of these strains in clinics in the USA and Europe presents an ever-increasing risk, since these pathogenic microbes are not affected by any of the presently used chemotherapeutic agents. Moreover, a series of clinical strains of staphylococcus that are not sensitive to teikoplanin but retain sensitivity to vancomycin have been found. Mutant strains of staphylococcus resistant both to teikoplanin and to vancomycin have already been obtained under experimental conditions. There is an enormous risk of the possible transmission at the genetic level of resistance from enterococci to staphylococci, which are significantly more widespread in clinics than enterococci. This may lead in medicine to the situation that existed before the antibiotic era, when sepsis or other diseases caused by multiresistant bacteria led to the rapid death of the patient.

One of the most effective ways of preventing the approaching catastrophe is the deliberate creation of products of a new generation, active against the multiresistant pathogenic microbes and, primarily, staphylococci and enterococci.

The molecular principles of the resistance to antibiotics of this group have been studied quite well. In the glycopeptide-resistant enterococci the C-terminal -Lys-D-Ala-D-Ala peptide fragment is replaced by the depsipeptide fragment -Lys-D-Ala-D-Lac (Fig. 1). This makes it impossible to form one of the five hydrogen bonds in the antibiotic-target complex, which leads to a significant decrease in its stability. It was shown on model peptides that the binding constant of vancomycin with diAcLys-D-Ala-D-Lac is three orders of magnitude lower (102 M^{-1}) than that of diAcLys-D-Ala-D-Ala (105 M^{-1}) [6], which correlates with the MSC (minimum suppressing concentration) for sensitive and resistant enterococci. (Usually the range of MSC amounts to 0.25-2 μg/ml for strains sensitive to vancomycin and 64-128 μg/ml for resistant strains.)

For a long time the role of the carbohydrate part of dalbaheptides in the mechanism of the antibacterial action remained unknown. The carbohydrates are undoubtedly involved in the transport processes of the antibiotics, but in recent years a more important function was discovered. It was shown that the presence of an additional amino sugar in eremomycin and in the antibiotic LY264826 compared with vancomycin makes it possible for dimers of these antibiotics to form [7]. The dimers are formed through the hydrogen bonds of the CO and OH groups that do not participate in interaction with the ligand. The dimer is of the "head-to-tail" type as shown in Fig. 2. The most stable dimers in water at pH 7 are given by eremomycin ($K_{dim} = 3 \times 10^6$ M^{-1} and chloroeremomycin ($K_{dim} = 1.8 \times 10^5$ M^{-1}) (according to NMR spectroscopy). We determined the value of Kdim for eremomycin in a 5 mM

TABLE 1. The Equilibrium Constant of the Dimerization of Dalbaheptides According to Data from NMR and ESI-MS [8]

Antibiotic	Relative degree of dimerization	% of dimer	% of monomer	K_{dim} (M^{-1}, ESI-MS data)	K_{dim} (M^{-1}), NMR data
Eremomycin	+++	88	12	1×10^6	3×10^6
Vancomycin	++	22	78	1200	700
Ristomycin	+	9	91	360	300

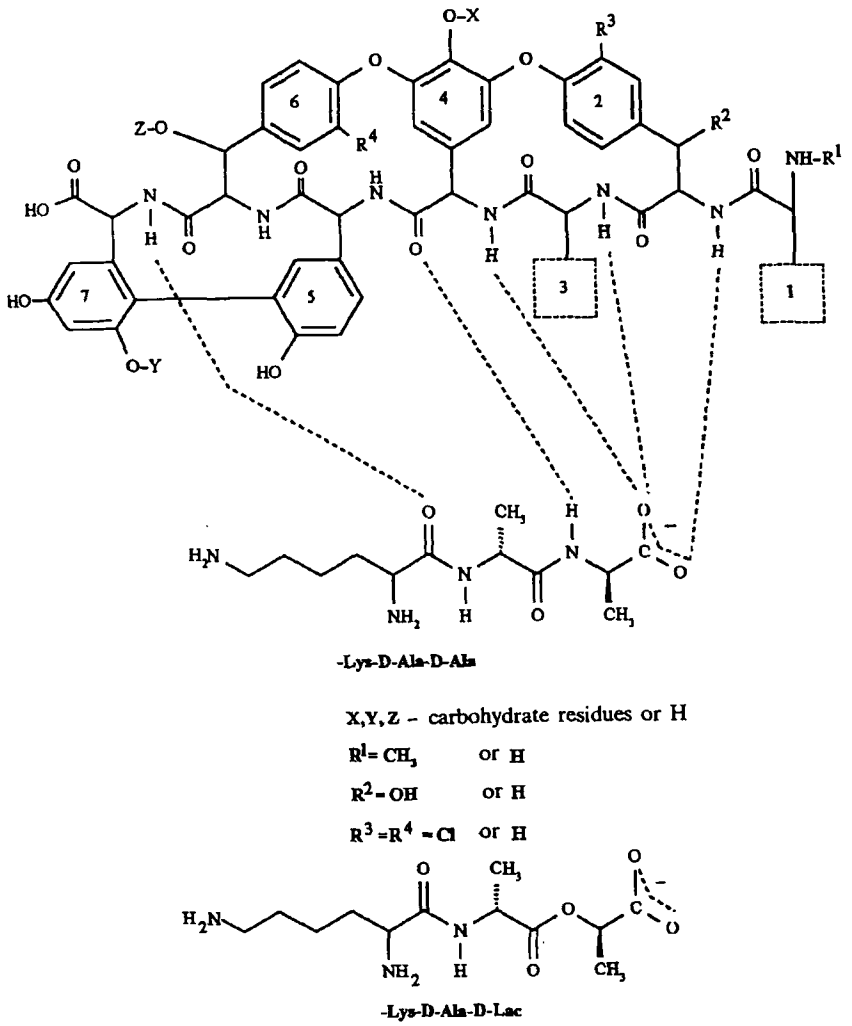


Fig. 1. A model of the complex of the glycopeptide with the C-terminal fragment -Lys-D-Ala-D-Ala of peptidoglycan in sensitive bacteria and with the Lys-D-Ala-D-Lac fragment.

solution of $AcONH_4$ at pH 5.1 ($K_{dim} = 1 \times 10^6\ M^{-1}$) by electro-spray mass spectrometry (ESI-MS). Vancomycin and ristomycin are considerably inferior to these antibiotics in their ability to dimerize. The dimeric antibiotic–antibiotic complexes become more and more stable with the addition of one or two molecules of the ligand; thus, K_{dim} with the ligand for eremomycin and chloroeremomycin is increased by two orders of magnitude. The addition of a second ligand is less favorable energetically than the first. Table 1 shows the ability of some dalbaheptides to undergo dimerization. However, there is no direct correlation between the ability of the glycopeptides to undergo dimerization and the level of antibacterial activity.

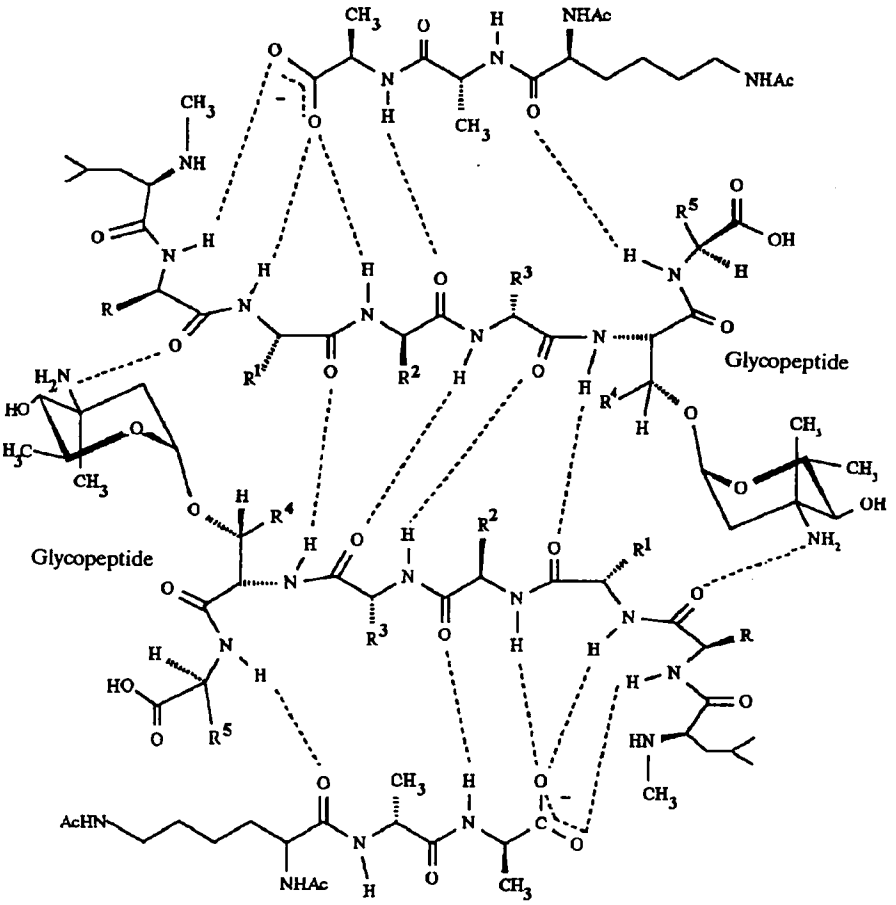


Fig. 2. The formation of the dimer of some glycopeptide antibiotics and its reaction with two molecules of AcLys(Ac)-D-Ala-D-Ala.

It is assumed that the natural antibiotics can react with the target -Lys-D-Ala-D-Ala either as monomers (vancomycin, the aglycone of teikoplanin) or as dimers (eremomycin, LY264826). These two models of interaction are shown schematically in Figs. 3a and 3b [9]. Compared with the monomers the dimers form a more stable antibiotic–target complex, and this leads to an increase in the antibacterial activity.

Figure 3 shows another possible mechanism for the stabilization of the antibiotic–target complex. It involves interaction of the lipophilic (acyl) fragment of certain dalbaheptides incapable of dimerization (e.g., teikoplanin or A-40926) with the membrane elements of the cell wall of the bacteria, at which the synthesis of the peptidoglycan fragment is realized.

The mechanism of the antibacterial action of the dalbaheptides and the genetic principles of resistance to them were examined in detail in the reviews [10, 11].

There are several approaches to the production of dalbaheptides active toward glycopeptide-resistant bacteria. The rational approach is based on modification of the sections of the molecule (the CONH fragments of the peptide core) that participate directly in the formation of the antibiotic–ligand complex. The most practical way of producing derivatives of such type is total synthesis. Substitution of the amino acids in the peptide core must be called a semiempirical and not a rational approach. However, the probability of changing the spectrum of antibacterial activity in this case is fairly high, since the molecule of the antibiotic is modified in the sections that interact with the target. Finally, there is an empirical approach—modification of the functional groups of the antibiotic not taking part in the formation of a complex with the target. This method of modification is easier to realize, and it has at present led to a series of derivatives active toward both glycopeptide-sensitive and glycopeptide-resistant microorganisms. In this case the ability of the antibiotic to interact with the cell wall of the bacteria is probably increased, and this suppresses the synthesis of the peptidoglycan, whereas binding to the D-Ala-D-Lac ligand is not increased. The

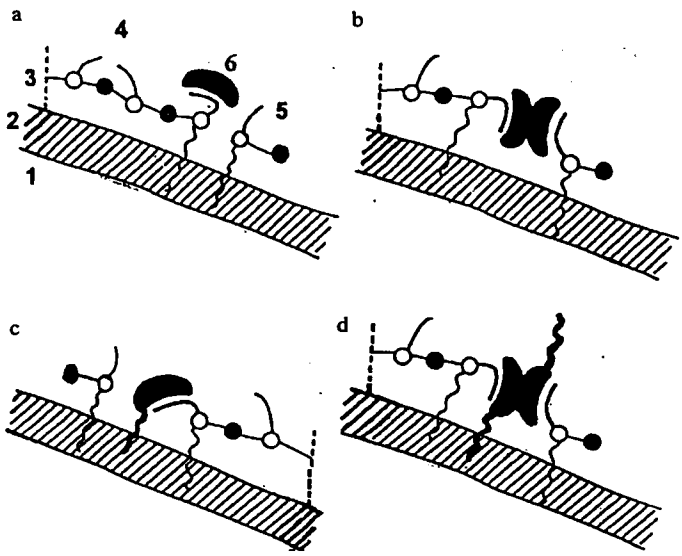


Fig. 3. The proposed conditional mechanisms of the interaction of glycopeptide antibiotics and their derivatives with the target -Lys-D-Ala-D-Ala (a-d): 1) Cytoplasm; 2) cytoplasmatic membrane; 3) bacterial wall; 4) growing peptidoglycan; 5) disaccharidepentapeptide fragment; 6) antibiotic.

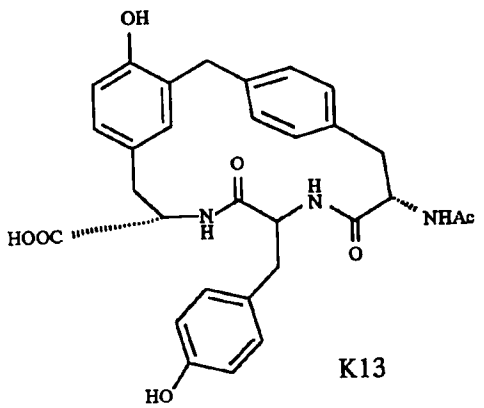
discovery of a structure–activity relation and the interpretation of the mechanism of the action of such modified antibiotics will make this approach rational.

2. THE SYNTHESIS OF GLYCOPEPTIDE ANTIBIOTICS

The Strategy of Total Synthesis

Two approaches have been used for the synthesis of the glycopeptide core of antibiotics. The first involves the production of a linear peptide followed by the formation of biaryl ethers as the key step of cyclization. The second approach involves the formation of functional biaryl ethers followed by macrolactamization (construction of the peptide link). This method is considerably rarer than the first but leads to positive results.

Cyclization is difficult when it is accompanied by a decrease of entropy and by an increase of the strain due to the formation of the rings. In the case of the macrocyclic structures of glycopeptides the rate of closure of the bifunctional chain into a ring is determined to a considerable degree by which conformer predominates in the initial noncyclic compound. The spatial approach of the aromatic rings, essential for closure of the macrocycle, is favored by a series of factors: π–π-interaction of the aromatic rings with electron-donating and electron-withdrawing substituents; intramolecular hydrogen bonds; the presence of D-amino acids or glycine, promoting the "bent" and not the β-folded conformation of the peptide, in the peptide chain. The last requirement proved not so important, since macrocyclization



K13

was successfully realized with the formation of the antibiotic K-13, consisting only of L-amino acids [12, 13]. The preorganization of the precursor of the cyclization product was called the intramolecular recognition phenomenon [14].

The construction of peptide fragments by classical methods [the use of EDC (N-3-dimethylaminopropyl-N′-ethylcarbodiimide), HOBt (1-hydroxybenzotriazole), triethylamine] does not present great problems at the present time. Certain complications were due only to the correct choice of initial reagents (aromatic acids) and to the presence of phenylglycine sensitive to racemization. The main problem in the total synthesis of glycopeptides was the development of cyclization methods. Three requirements have to be considered: 1) Mild reaction conditions on account of the presence of sensitive functional groups; 2) the introduction of a single substituent at the *ortho* position of the Ar–O–Ar bridge; 3) the attainment of good yields of the cyclization products; 4) the creation of a strained biaryl structure; 5) control of the atropisomerism (conformational isomerism) during the formation of the fragments of biaryl ethers included in the macroheterocycle [15].

The last point requires some explanation. In the glycopeptide antibiotics of the vancomycin group rotation about the Ar–O bond in the 16-membered macrocycles M(2-O-4) and M(4-O-6) and the C–C bond in the macrocycle M(5-7) and also the C–O bond in the macrocycle M(1-O-3) is hindered. The presence of one chlorine atom each in aromatic rings 2 and 6 makes it possible for four atropodiastereoisomers to exist only for the system with two macrocycles M(2-O-4) and M(4-O-6). In this connection the formation of an atropoisomer identical with the natural compound presents an enormous problem.

A significant contribution to the development of the synthetic chemistry of glycopeptides has been made by condensation methods. Some are based on intramolecular aromatic substitution S_NAr using aromatic derivatives in which nitro or other electron-withdrawing groups are situated at the *o* position to the halogen that reacts with the nucleophilic OH group of the neighboring phenylglycine residue (in the presence of CsF, K_2CO_3, etc.) [16-20]. Others are based on the oxidation of derivatives of phenols by thallium(III) salts [21-23] and also on the effective cycloetherification method of Sudzuki, in which $Pd(PPh_3)_4$ is used for the formation of the Ar–Ar bond [24].

Synthesis of the *seco*-Aglycone of Vancomycin

Researches into the total synthesis of glycopeptide antibiotics were conducted in the middle of the eighties. As the methods of cyclization developed, syntheses of ever more complex model glycopeptide systems were realized. Thus, a method was developed for the synthesis of the model 16-membered M(4-O-6) glycopeptide ring [25], the bicyclic M(2-O-4)(4-O-6) fragment of vancomycin was synthesized [22], and a series of other compounds were obtained [26, 27]. A significant contribution in this region was made by the discovery by Yamamura's group of an elegant method involving the oxidation of aryl rings each having one hydroxyl and two halogen substituents by thallium(III) nitrate. The possibilities of this method were demonstrated in the total synthesis of the antibiotic K-13 [28].

Using the phenol oxidation method as the key step in the construction of bicyclic diaryl ethers, Yamamura and coworkers obtained the *seco*-aglycone of vancomycin (Ia) and its tetrachloro derivative (Ib) (Scheme 1) [29]. These compounds have a full heptapeptide chain and two characteristic biaryl ether bridges.

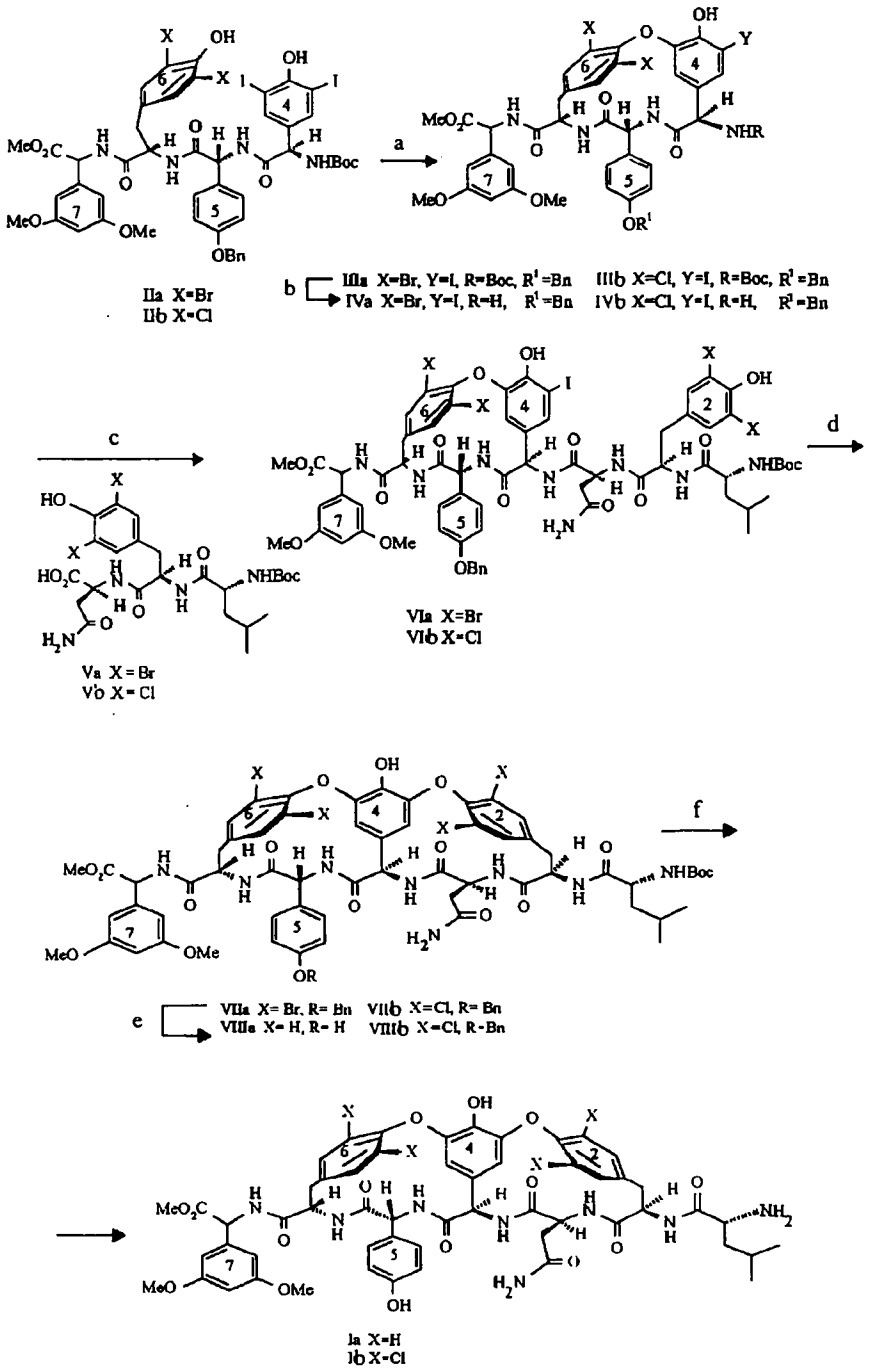
The tetrapeptide (IIa) was obtained by a peptide synthesis. Thallium(III) nitrate was used as substrate for the oxidative linkage of the phenyl rings 4 and 6, and the cyclic product (IIIa) was obtained (42%). After removal of the Boc protecting group compound (IVa) was condensed with the tripeptide (Va), and the heptapeptide (VIa) was obtained (59%). The final macrocyclization was realized in the same way as for compound (IIa), and the bicyclic derivative (VIIa) was obtained with a 35% yield. Its dehalogenation (H_2–Pd/C) followed by removal of the Boc protection led to the dechloro-*seco*-aglycone of vancomycin (Ia) (with the fifth and seventh amino acids unlocked in space). The linkage of the residues of these aromatic amino acids with the formation of the macrocycle M(5-7) proved an extremely complicated task.

The synthesis of the tetrachloro-*seco*-aglycone of vancomycin (Ib) was realized in a similar way, using the dichloro derivatives instead of the derivatives containing bromine at the initial stages of the peptide synthesis (Scheme 1).

Synthesis of the Bicyclic M(2-O-4)(4-O-6) Fragment of Vancomycin

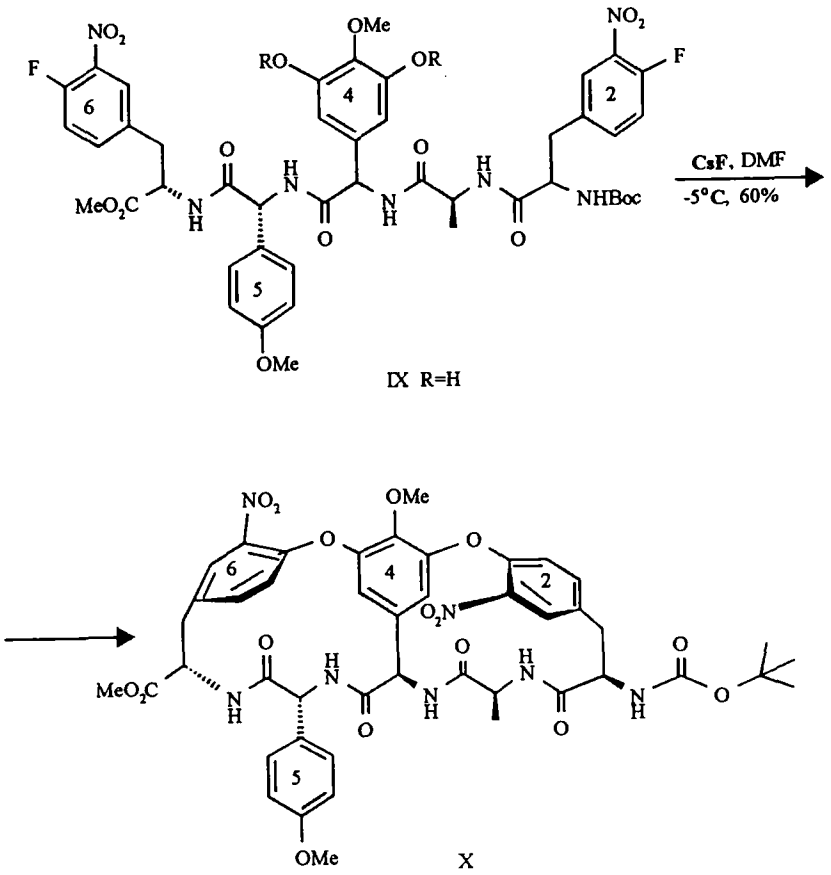
The synthesis of analogs of vancomycin type has been carried out simultaneously by many investigators. In contrast to the method developed by Yamamura, Zhu and coworkers used a different approach to the creation of the model bicyclic M(2-O-4)(4-6) structure of vancomycin. The linear pentapeptide skeleton had been synthesized

Scheme 1



Reagents and conditions: a) $Tl(NO_3)_3 \cdot 3H_2O$, THF/MeOH/$CH(OMe)_3$ (8 : 1 : 1) (IIIa: 42%, IIIb: 40%); b) CF_3COOH (IVa: 81%, IVb: 61%); c) N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC), 1-hydroxybenzotriazole (HOBt), DMFA (VIa: 59%, VIb: 53%); d) $Tl(NO_3)_3 \times 3H_2O$, THF/MeOH/$CH(OMe)_3$ (8 : 1 : 1); then Zn, AcOH (VIIa: 53%, VIIb 40%); e) H_2—Pd/C, MeOH (VIIIa; 40%, VIIIb; 40%); f) CF_3COOH (Ia: 61%, Ib: 76%)

Scheme 2

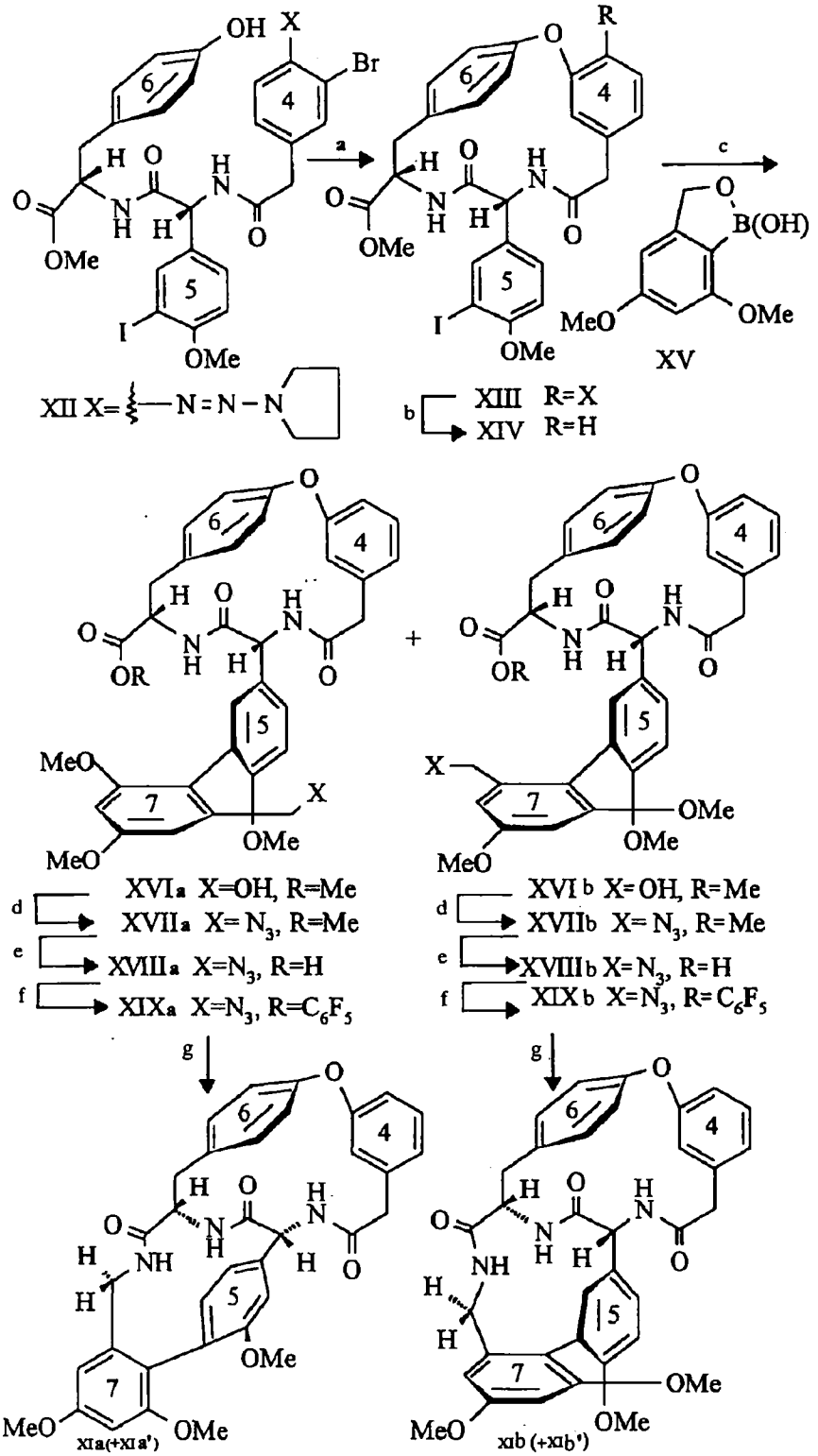


earlier, and the concluding stage in the production of this molecule was the formation of the two Ar–O–Ar ether bonds (Scheme 2) [30]. The macrocyclization of the pentapeptide (IX) was realized by using the intramolecular S_NAr reaction twice. An important feature of this reaction apart from the mild conditions and the high yields is the fact that it makes it possible to control the atropodiastereoselectivity during macrocyclization. Cyclization was conducted at -5°C in DMFA with CsF as catalyst, and the single atropodiastereoisomer (X), not identical with the natural vancomycin isomer, was isolated (60%). When the reaction conditions (mostly the temperature) were changed, all four possible atropostereoisomers were obtained and separated [15].

Construction of the Bicyclic M(4-O-6)(5-7) Fragment of Glycopeptides

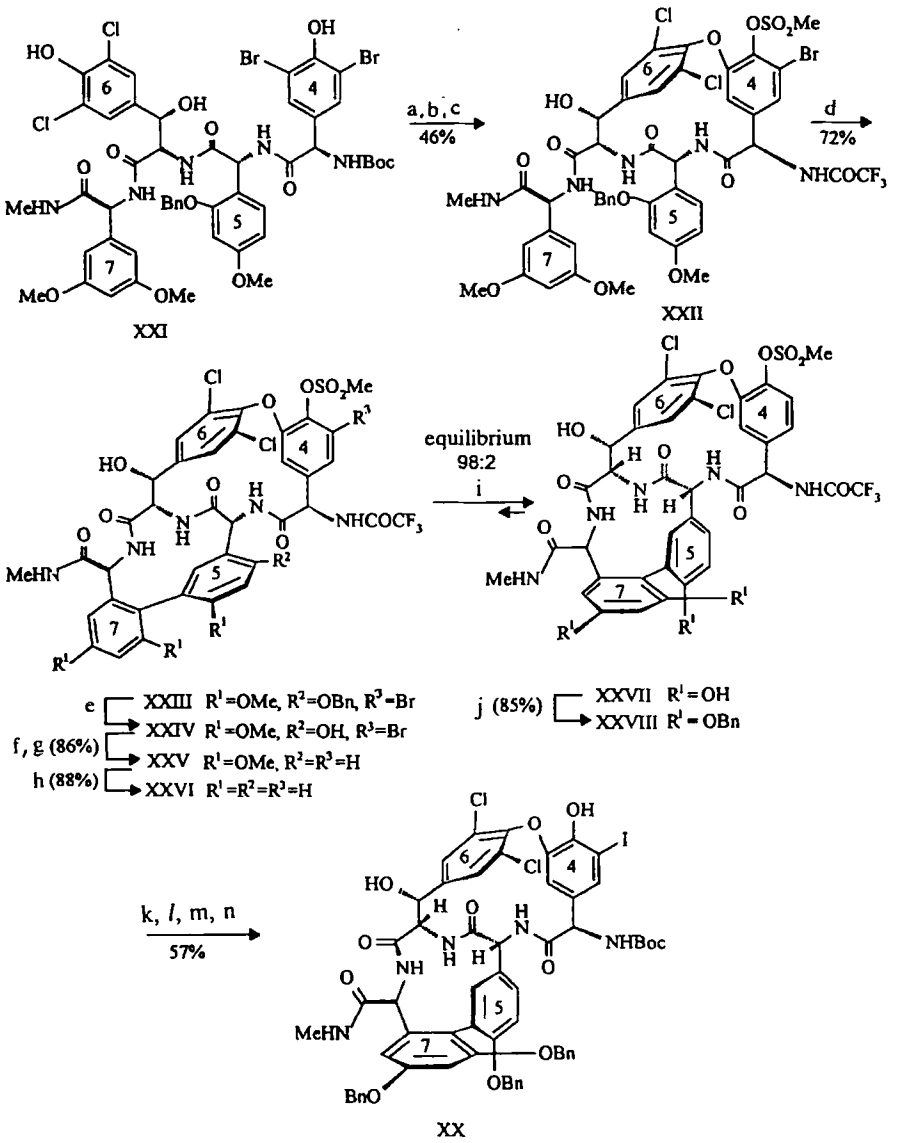
As mentioned above, the formation of the strained biaryl M(5-7) structure is one of the main problems in the total synthesis of glycopeptides [31, 32]. All attempts to create this macrocycle as a single system by lactamization were unsuccessful [33]. Nikolaou and coworkers showed that such a structure could only be produced in the presence of the already prepared M(4-O-6) fragment, which favors lactamization. They were able to obtain the bicyclic M(4-O-6)(5-O-7) model of glycopeptide antibiotics (XI), using the S_NAr reaction and also the Sudzuki coupling reaction followed by the formation of the peptide bond (Scheme 3) [24]. Unfortunately, these reactions do not exhibit atropostereoselectivity, and as a result complex mixtures of atropostereoisomers are formed. This makes it necessary to separate the isomers and significantly reduces the yields of the desired product. The structure of the atropostereoisomers was determined by NMR spectroscopic methods. The cyclization of the tripeptide (XII), which contains the triazene residue –N=N–N(CH_2)4 in phenyl ring 4 at the *o* position to the leaving group, in the presence of K_2CO_3–CuBr·SMe_2 led to the macrocycle (XIII) with a 67% yield. The reaction was accompanied by epimerization of the phenylglycine derivative (10% comprised the unnatural epimer). Compound (XIII) was separated by chro-

Scheme 3



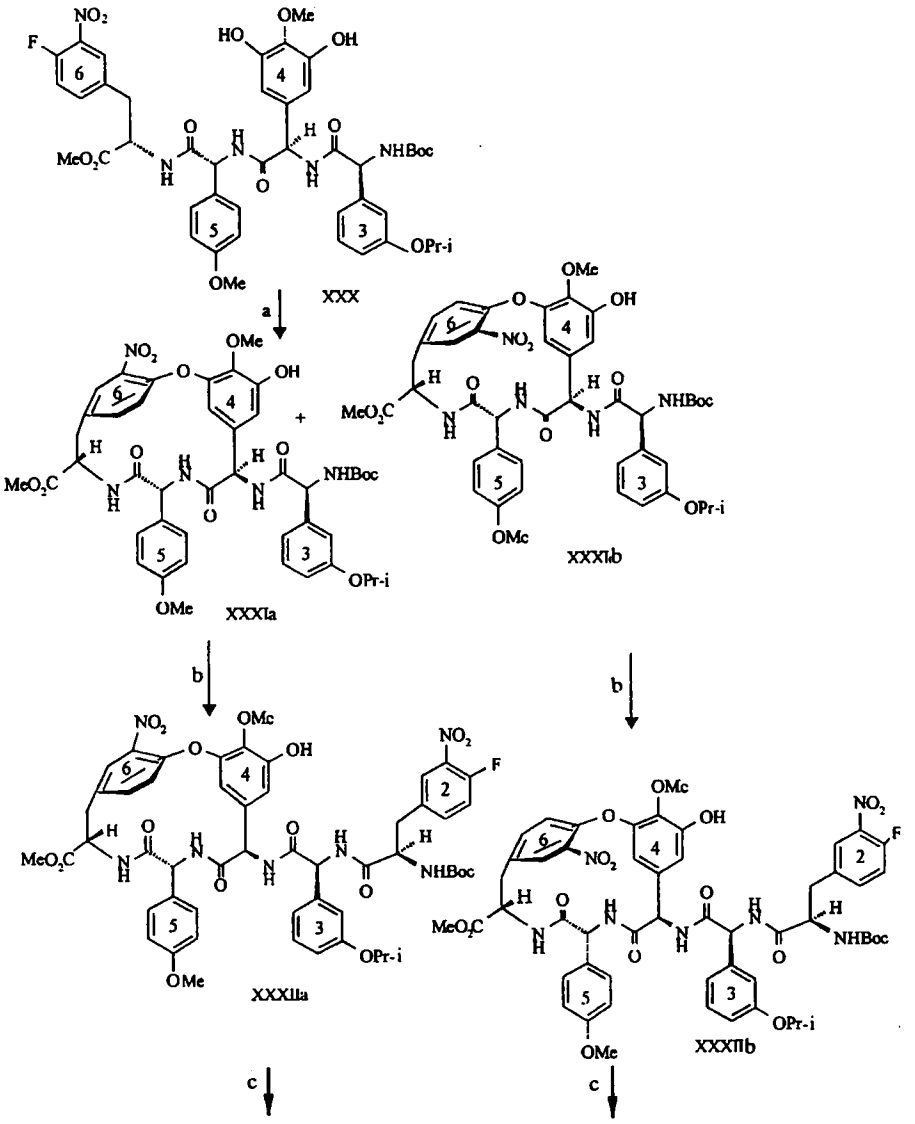
Reagents and conditions: a) $CuBr \cdot SMe_2$, K_2CO_3, pyridine, MeCN, 36 h, 67%; b) CF_3COOH, Cu_2O, MeCN/THF/H_2O, 1 h, 90%; c) $Pd(PPh_3)_4$, Na_2CO_3, MeOH/toluene/H_2O, 90 °C, 2 h, 80%; d) HN_3, DEAD (diethyl azodicarboxylate), PPh_3, THF, 0 — 20 °C, 1 h, 69%; e) LiOH, THF/H_2O, 0 °C, 0.5 h, 100%; f) C_6F_5OH, DCC (dicyclohexylcarbodiimide), DMAP (4-dimethylaminopyridine), CH_2Cl_2, 25 °C, 1 h; g) 4-pyrrolidinopyridine, 10% Pd/C, dioxane/EtOH/ cyclohexane, 90 °C, 5 h, 30% from XVIIIa

Scheme 4



Reagents and conditions: a) $Tl(NO_3 \cdot 3H_2O$, pyridine, THF/MeOH, 0 °C, then $CrCl_2$, THF/MeOH, 0 °C; b) $MeSO_2Cl$, *i*-Pr_2NEt, THF, 0 °C; c) CF_3COOH, Me_2S, CH_2Cl_2, 0 °C; then $(CF_3CO)_2O$, 2,6-lutidine, CH_2Cl_2, 0 °C; d) VOF_3, $BF_3 \cdot OEt_2$, $AgBF_4$, CF_3COOH, 0 °C; e) Zn; f) $PhNTf_2$, K_2CO_3, DMFA, THF/MeOH, 0 °C; g) $Pd(PPh_3)_2Cl_2$, Et_3N, HCOOH, DMFA, 75 °C; h) $AlBr_3$, NaI, $Cl(CH_2)_2Cl$; i) MeOH, 55 °C, 96h; j) BnBr, Cs_2CO_3, Bu_4NI, DMFA, 0 °C; k) $NaBH_4$, EtOH, 0 °C; *l*) MeMgCl, THF, 0 °C; m) Boc_2O, $NaHCO_3$, dioxane/H_2O, 20 °C; n) N-iodosuccinimide, DMFA, 20 °C

Scheme 5

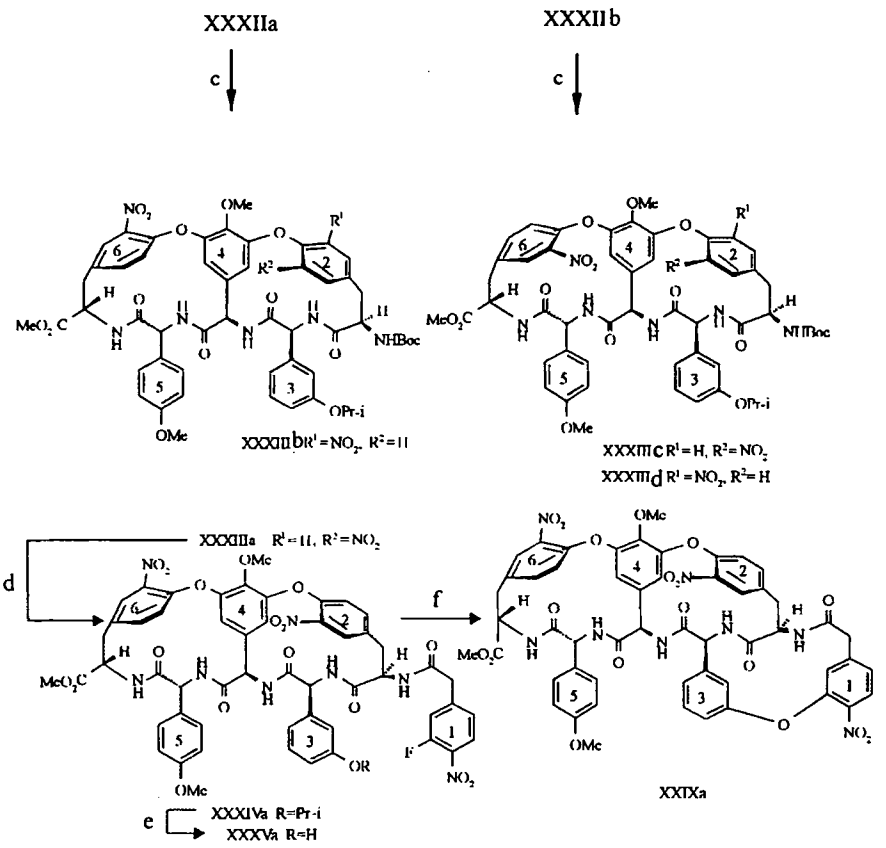


matography, the triazene residue was removed by the action of CF_3COOH–Cu_2O, and the desired M(4-O-6) fragment (XIV) was obtained (90%). The product was used for further synthesis.

Under the conditions of the Sudzuki reaction [$Pd(Ph_3P)4$ and Na_2CO_3] the macrocyclic derivative (XIV) was condensed with the phenylboronic acid derivative (XV) with the formation of a mixture of two separable atropoisomers (XVIa) and (XVIb) (1:1) with an overall yield of 80%. The action of ammonia, diethyl azodicarboxylate (DEAD), and triphenylphosphine on compound (XVIa) gave the azide (XVIIa) (yield 69%), which was treated with lithium hydroxide to hydrolyze the C-terminal methyl ether. The hydroxyl group of the compound (XVIIIa) obtained in this way was converted into the pentafluorophenyl ether (XIXa), the lactamization of which led to the formation of the cyclic products (XIa) and (XIa′) (a 1:2 mixture of epimers with respect to C-6) with an overall yield of 30%. The bicyclic compounds (XIb) and (XIb′) were obtained similarly starting from (XVIb). Compounds (XIa) and (XIb) are similar in the structure of the left part of the "binding pocket" of the glycopeptides, but they do not contain the terminal COOH and NH_2 groups. The synthesis of these compounds is a rare example of cyclization where the formation of the Ar—Ar bond in the macrocycle precedes ring closure on account of the formation of the peptide bond.

The analogous M(4-O-6)(5-7) bicyclic system (XX) was obtained by Evans and coworkers [34]. The starting compounds were aromatic amino acids, which were linked successively into the tetrapeptide (XXI) by the standard

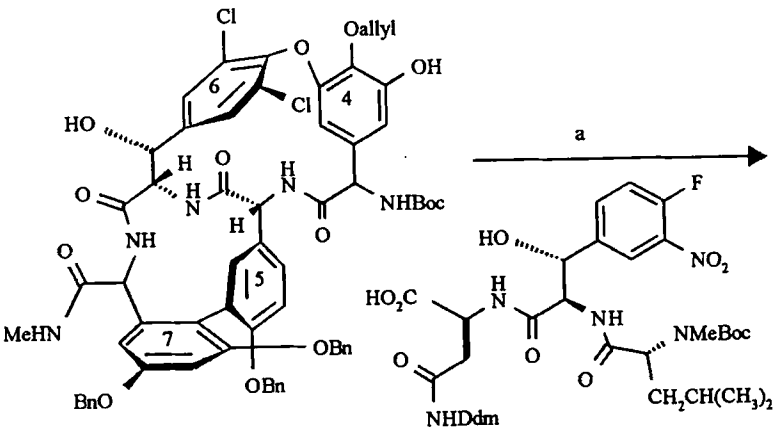
Scheme 5 (Continued)



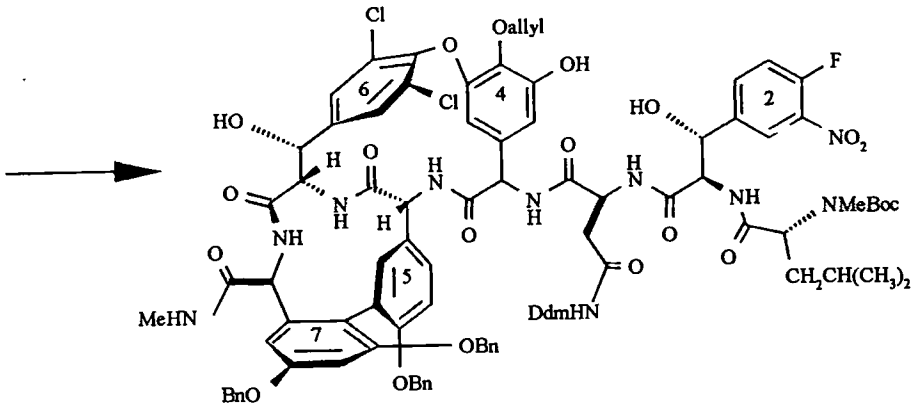
Reagents and conditions: a) CsF, DMFA, 5 °C, 20 h, 60%; b) HCl/MeCN, 20 °C, 1 h, then D-N-Boc-3-fluoronitrophenylalanine pentafluorophenyl ester, 5 °C, 5 h, 60%; c) analogous conditions for *a*, 60%; d) HCl/MeCN, 20 °C, 1 h, then diphenylphosphorylazine (DPPA), DMFA, Et_3N, 3-fluoro-4-nitrophenylacetic acid, 0 — 20 °C, 6 h; e) BCl_3, CH_2Cl_2, 0 °C, 7 h; f) CSF, DMFA, molecular sieves, 20 °C, 3 h

methods of peptide synthesis. The desired bicyclic system (XX) was subsequently obtained by using oxidative cyclization reactions (Scheme 4). Thus, the formation of the M(4-O-6) macrocycle (XXII) was realized by the action of $Tl(NO_3)_3$ with a yield of 70%. The phenolic hydroxyl in ring 4 of this compound was protected by an acid-resistant mesylate residue in order to intensify the acidic characteristics of this ring in relation to rings 5 and 7 and make it possible subsequently to modify this ring selectively. Oxidative cyclization of (XXII) (VOF_3, $BF_3{\cdot}OEt_2$, $AgBF_4$, CF_3COOH) led to the highly strained bicyclic tetrapeptide (XXIII). It should be noted that the unnatural (*R*)-atropostereoisomer is formed preferentially under these conditions. Then, after removal of the protecting benzyl group in ring 5 and the bromine atom in ring 4 compound (XXV) was obtained (86%). It was treated with aluminum bromide and sodium iodide in order to deblock the protecting methoxy groups. Conditions were selected for the quantitative conversion of the unnatural atropostereoisomer into the required (*S*)-atropostereoisomer (XXVII) (methanol, 55°C, 96 h), the structure of which was confirmed by 1H NMR experiments. The bicyclic tetrapeptide (XXVII) was modified in such a way that it was subsequently possible to obtain the aglycone of orienticin C, the synthesis of which is described below. Thus, the three phenolic hydroxyls in compound (XXVII) were protected in the form of benzyl ethers without appreciable epimerization. By reductive removal of the trifluoroacetamide ($NaBH_4$) and also the mesyl groups (MeMgCl) from compound (XXVIII) and subsequent *tert*-butyloxycarbonylation (Boc_2O, $NaHCO_3$) of the amino group it was possible to iodinate ring 4 selectively, using N-iodosuccinimide, and obtain the bicycle (XX) with a 57% yield.

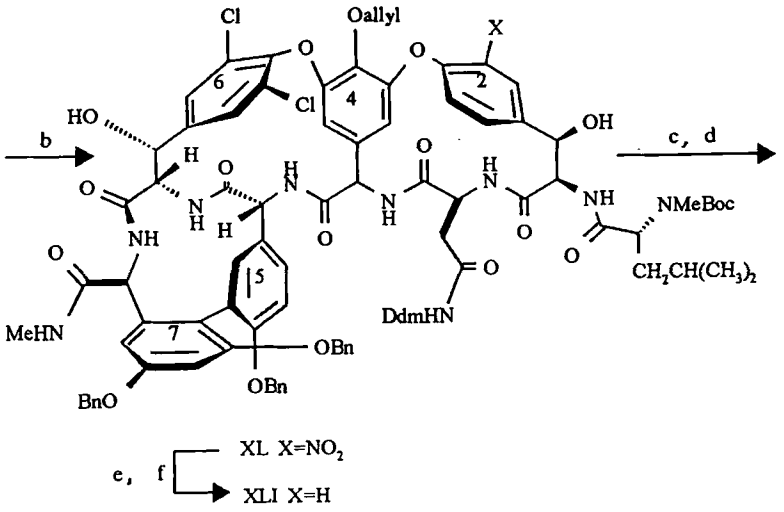
Scheme 6



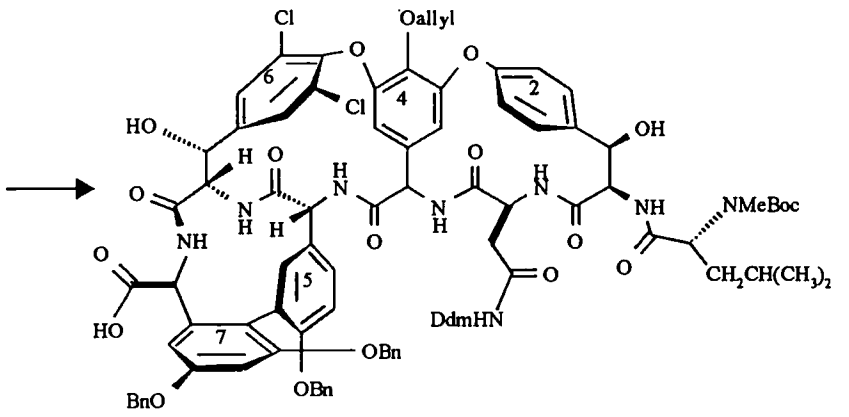
XXXVII XXXVIII



XXXIX

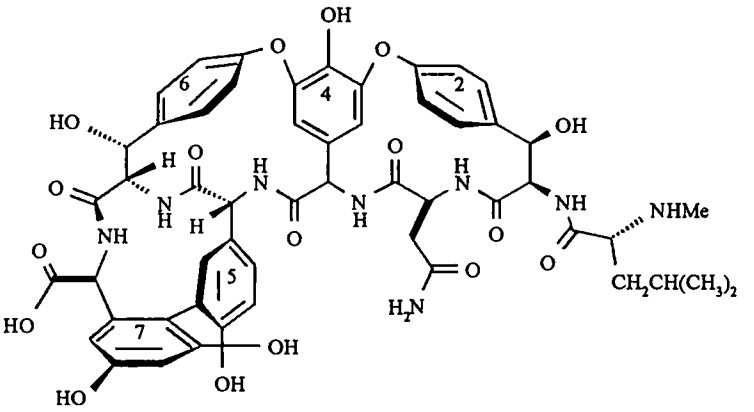


Scheme 6 (continued)



XLII

g, h, i



XXXVI

Reagents and conditions: a) CF_3COOH, Me_2S, CH_2Cl_2, 0 °C, ; then 3, EDC, HOAt, THF, 0 °C, 75%; b) CsF, DMSO, 20 °C, 90%; c) Zn, HOAc, EtOH, 40 °C; d) H_3PO_4, $NaNO_2$, THF/H_2O, Cu_2O, 0 °C 85%; e) N_2O_4, NaOAc, CH_2Cl_2/CH_3CN, 0 °C; f) H_2O_2, LiOH, THF/H_2O, 0 °C, 46%; g) $Pd(PPh_3)_4$, morpholine THF, 20 °C; h) 10% Pd/C, H_2, MeOH, 20 °C; i) CF_3COOH, Me_2S, CH_2Cl_2, 20 °C

Synthesis of the Tricyclic Fragment M(1-O-3)(2-O-4)(4-O-6) of Teikoplanin

As already mentioned, antibiotics of the teikoplanin group, unlike antibiotics of the vancomycin group, contain four macrocycles. The presence of the additional fourth ring, formed by the first and third amino acids, considerably complicates the synthesis. At the initial stages of researches in this region only small fragments of the molecule were obtained, close in structure to K-13 [18, 35] or to the fragment M(1-O-3)(4-O-6) of natural antibiotics [36].

Using the previously obtained results and a considered choice of reaction components, Zhu and coworkers proposed the following scheme for the production of compound (XXIX) (Scheme 5) [37].

From the tetrapeptide (XXX) under the conditions of an intramolecular S_NAr reaction a 16-membered macrocycle was formed as a mixture of two atropoisomers (XXXIa) and (XXXIb) (1:1) (60%), which were separated. Deblocking of each of them (HCl, MeCN) followed by reaction with N-Boc-4-fluoro-3-nitro-D-phenylalanine pen-

tafluorophenyl ester led to the pentapeptides (XXXIIa) and (XXXIIb) with 50-60% yields. Cycloetherification of (XXXIIIa) with CsF gave again a mixture of the two atropoisomers (XXXIIIa) and (XXXIIIb) (1:1) with bicyclic structure. The bicycles (XXXIIIc) and (XXXIIId) are also formed from compound (XXXIIb) under the same conditions as a mixture of two atropoisomers (1:1.4). Removal of the Boc group from compound (XXXIIIa) (HCl, MeCN) followed by the introduction of the 3-fluoro-4-nitrophenylacetic acid residue gave compound (XXXIVa), from which after removal of the isopropyl protecting group (BCl_3, CH_2Cl_2) compound (XXXVa) was obtained. Cycloetherification of (XXXVa) (CsF, DMFA) led to the tricyclic M(1-O-3)(2-O-4)(4-O-6) analog of teikoplanin (XXIXa). The overall yield of the reaction, starting from compound (XXX), amounted to 5-7%. The tricyclic compound (XXIXb) was obtained from (XXXIIIb) according to the same scheme. The main difference between these macrocycles (XXIXa) and (XXIXb) and the aglycone of teikoplanin lies in the absence of the seventh amino acid. A complete synthetic analog of the aglycone of teikoplanin has not yet been obtained, but in view of already developed approaches to closure of the M(5-7) macrocycle [23] its total synthesis can be expected in the near future.

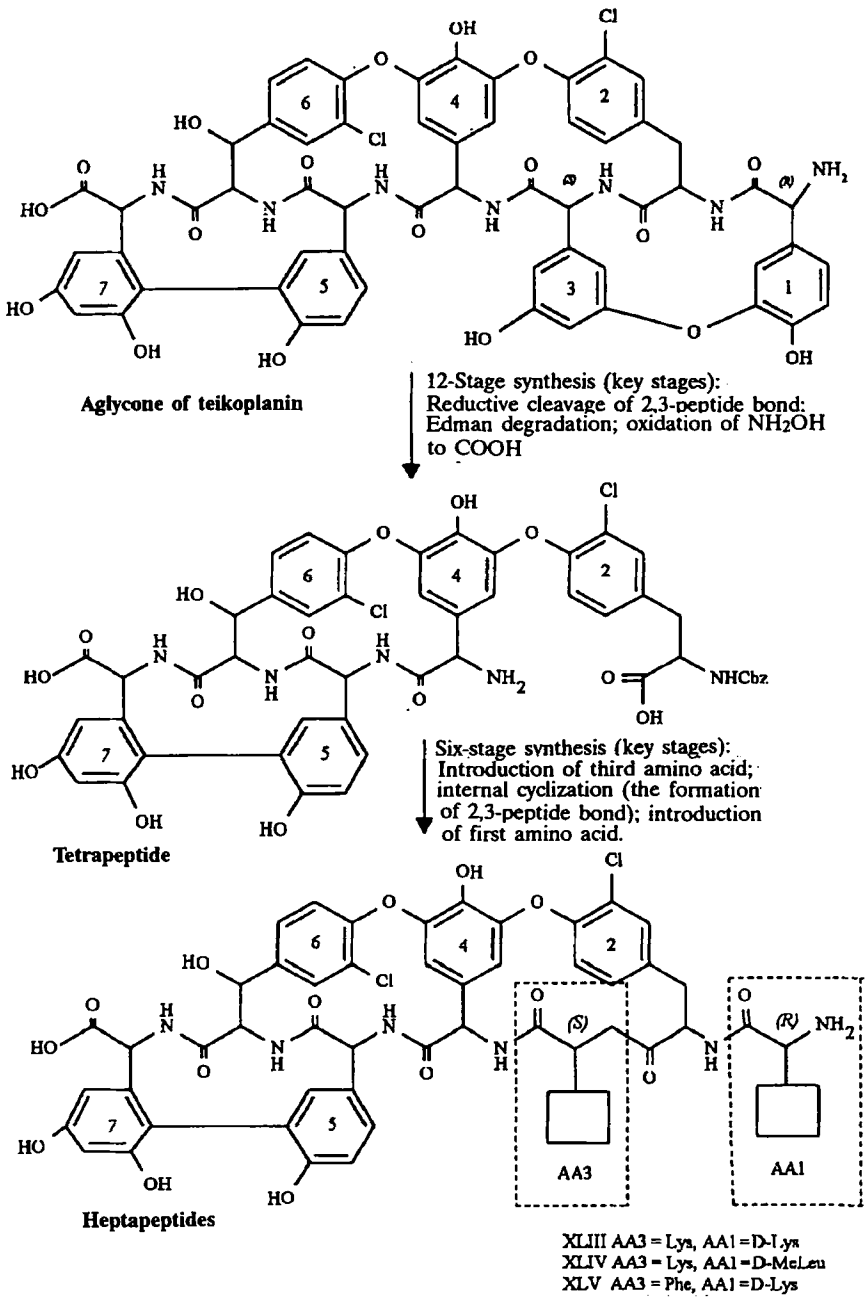
Total Synthesis of the Aglycone of Orienticin C

Outstanding results on the total synthesis of glycopeptide antibiotics were obtained by Evans and coworkers. Until recently the researches of this group were directed at the effective synthesis of the essential amino acid structures [38-40] and also at the choice of conditions for oxidative cyclization by means of thallium(III) salts to produce the M(2-O-4)(4-O-6) bicyclic system [22]; at the intramolecular vanadium-dependent construction of the biaryl strained M(5-7) ring [23, 31]; at opening of the M(2-O-4) ring, using intramolecular S_NAr substitution [41]. The successful combination of all these methods led to the first total synthesis of the aglycone of orienticin C (XXXVI). Prior developments had secured the choice of the key initial synthons (XXXVII) and (XXXVIII) for this synthesis. The bicyclic tetrapeptide (XXXVII) was linked to the N-terminal tripeptide (XXXVIII) (Scheme 6) [42], containing the 4′,4′-dimethoxydiphenylmethylasparagine residue. The choice of protective group was due to the fact that it was necessary to protect the asparagine residue during modification of the C-terminal N-methylamine at the end at the last stage of the synthesis and also to its favorable effect in suppressing the epimerization and rearrangement of the aspartate to the isoaspartate in the ring [43]. Relatively standard conditions were used for the peptide synthesis of the deblocked (XXXVII) with compound (XXXVIII) (EDC, THF, 0°C). However, instead of the previously employed HOBt the recently proposed 1-hydroxy-7-azabenzotriazole (HOAt) [44] was used in order to prevent epimerization, which is possible during the formation of the intermediate O-acylisourea derivative. The cyclization of the heptapeptide (XXXIX) was conducted by the action of CsF in DMSO, and compound (XL) was obtained as a mixture (7:1) of two atropoisomers. After removal of the nitro group by reduction, diazotization, and decomposition of the diazo derivative compound (XLI) was obtained. Selective nitrosation (N_2O_4, NaOAc, CH_2Cl_2, MeCN, 0°C) in the presence of other amide functional groups followed by hydrolysis with LiOH was used for the transformation of the N-methylamide, located at the C-terminal section of the molecule. The acid (XLII) was obtained in this way.

The aglycone of orienticin C (XXXVI) was obtained by the successive removal of the remaining protecting groups: allyl by the action of $Pd(PPh_3)_4$ in morpholine, benzyl by hydrogenolysis over Pd/C, and the acid-labile Boc and dimethoxydiphenylmethyl groups in trifluoroacetic acid. The hydrolysis of the two chlorine atoms in the sixth aromatic ring takes place simultaneously with the removal of the benzyl groups.

In the present review we are not concerned with aspects of the O-glycosylation of the aglycones of glycopeptides. The genes responsible for the biosynthesis of chloroeremomycin by a culture of *Amycolatopsis orientalis*, including the genes of three glycosyltransferases homologous with the two glycosyltransferases participating in the biosynthesis of vancomycin, were recently identified [45]. It was established that one of the genes is capable of synthesizing an enzyme that adds glucose to the heptapeptide core. *Streptomyces toyocaensis*, which produces the unglycosylated heptapeptide antibiotic A47934, after the introduction of cloned glycosyltransferases produced a new hybrid antibiotic — glucosyl-A47934 [46]. Thus, the problems of O-glycosylation of heptapeptides will be solved better by the methods of biotechnology. The expression of other genes, such as those responsible for the biosynthesis of aryl ethers, the β-hydroxylation of tyrosine, or the introduction of chlorine, may in the future be used for the modification of a different type of glycopeptides and the production of derivatives with valuable characteristics. The successful utilization of synthetic and biotechnical methods in the total synthesis of glycopeptide antibiotics will not only help toward an understanding of the details of the mechanism of their action but will also make it possible to construct new compounds that are clearly complementary to the resistant target.

Scheme 7



18-Stage synthesis of unnatural aglycones based on the aglycone of teikoplanin.

3. CHEMICAL MODIFICATION OF THE PEPTIDE CORE

As seen from Fig. 2, the first and third amino acids take direct part in the formation of the antibiotic—target complex. They form the walls of a hydrophobic pocket, in which the replacement of these amino acids by other amino acids or their analogs can lead to an increase in the stability of the antibiotic—target complex both in sensitive and in resistant bacteria.

The most suitable subjects for replacement of the first amino acid are glycopeptides with an aglycone of the vancomycin type. A series of unnatural analogs of these antibiotics were obtained from vancomycin [47] and the aglycone of eremomycin [48] by a three-stage synthesis, including elimination of the first amino acid by the Edman method, aminoacylation of the obtained hexapeptide, and removal of the protecting groups. The derivatives containing D-Lys or D-Ala as first amino acid have activity comparable with the activity of the initial antibiotics. However, the replacement of D-MeLeu by D-Trp or D-His in the aglycone of eremomycin led to the complete loss of activity.

The simultaneous replacement of the first and third amino acids proved considerably more difficult. It only became possible as a result of the discovery by Malabarba and coworkers of the unusual reductive cleavage of the 2,3-peptide bond [49, 50]. By means of this reaction a tetrapeptide was synthesized from the aglycone of teikoplanin in 12 stages (Scheme 7) [51]. This was the key compound in the synthesis of new unnatural glycopeptides with various amino acid residues at positions 1 and 3. Three unnatural aglycones of the vancomycin type (XLIII-XLV) with high activity toward sensitive bacteria were then obtained by a six-stage synthesis [52, 53]. Compounds (XLIV) and (XLV) exhibited activity toward glycopeptide-resistant enterococci, but this was insufficient to regard them as prospective products for clinical practice.

In spite of the large amount of synthetic work and some revealing results the total synthesis of glycopeptide antibiotics remains very laborious and costly. Considerably simpler and giving more promising results today is the empirical approach — chemical modification of dalbaheptides aimed at the transformation of fragments of the antibiotic molecules that do not take part in the formation of a complex with the target.

4. MODIFICATION OF FRAGMENTS OF GLYCOPEPTIDES NOT TAKING PART IN BONDING WITH THE TARGET

Dalbaheptides are complex polyfunctional amphoteric compounds soluble in DMSO, DMFA, methanol, water, and aqueous organic mixtures (1:1). The amphoteric properties are due to the presence of the phenolic hydroxyls, carboxyl, and amino groups. The problem of solubility in organic solvents, the presence of acid- and alkali-labile glycosidic bonds, and also the problem of the selectivity of the transformations substantially limit the possibilities of the chemical modification of dalbaheptides. Practically all the syntheses of the derivative of glycopeptide antibiotics require purification with the use of costly methods of multiple column ion-exchange chromatography, costly column chromatography on silylated silica gel, or preparative HPLC.

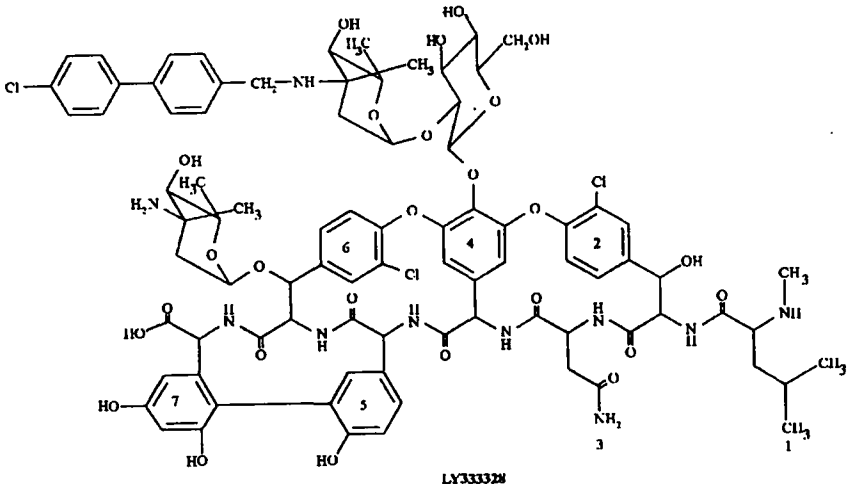
In spite of the listed difficulties, at the present time more than 1000 different derivatives of these antibiotics have been synthesized. They can be divided into five types: Deglycosylated derivatives; derivatives with respect to the phenolic hydroxyl groups, to the aromatic rings, and to the carboxyl and amino groups. This subdivision is arbitrary, since a large number of derivatives containing substituents at various positions simultaneously (di- and trimodified derivatives) were synthesized.

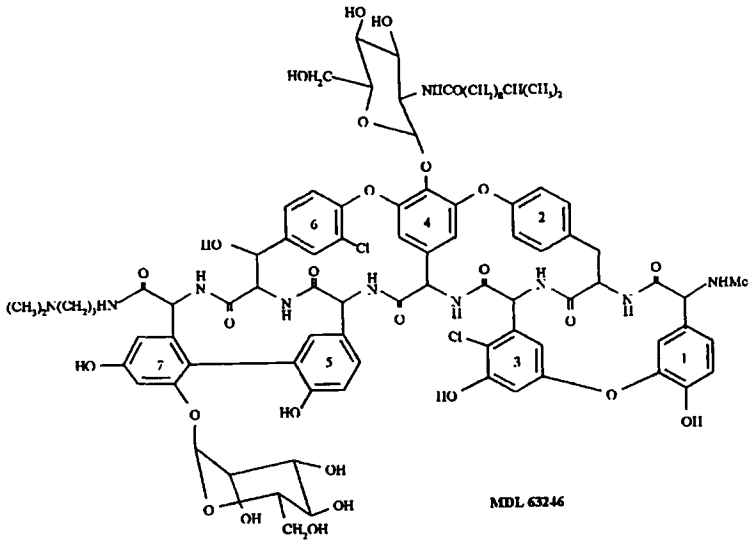
Almost all the researches on the chemical modification of dalbaheptides were carried out on the most important antibiotics shown in section 1. Reviews have been devoted to detailed discussion of these papers [54-59]. In the present review we only examine the most promising directions in the chemical modification of dalbaheptides: The synthesis of alkyl derivatives with respect to the amino groups of sugars, amides with respect to the carboxyl group of the seventh amino acid, and aminomethyl derivatives (Mannich bases) with respect to the aromatic ring of the seventh amino acid. These directions have made it possible at the present time to obtain a series of derivatives that are highly active toward both sensitive and resistant bacteria.

The first papers on the alkylation of the amino groups of sugars were presented 10 years ago, when the problem of resistance to dalbaheptides was not so acute as at present. A series of derivatives of vancomycin [60] and eremomycin [61, 62] with respect to the amino group of the sugar residue of the disaccharide branch were obtained by reductive alkylation by the respective aldehydes in the presence of $NaBH_3CN$.

A series of lipophilic derivatives of vancomycin exhibited high activity *in vivo*. Thus, the most active N-*p*-octyloxybenzylvancomycin surpassed vancomycin 4-9 times in effectiveness and had good pharmacokinetic characteristics. More recently, during investigation of these derivatives it was found that many of them (e.g., decyl, undecylenyl, *p*-chloro-, *p*-octyloxy-, or *p*-butyloxybenzyl derivatives) exhibit activity *in vitro* toward glycopeptide-resistant enterococci (MSC 4-128 μg/ml) [63]. Although the derivatives were not selected for clinical trials, the discovered structure—activity relationships enabled the same research group to obtain highly active N-alkyl derivatives of the antibiotic LY264826 [64-66].

More than 200 derivatives of LY264826 of this type were synthesized. Among them the most active *in vitro* with respect to glycopeptide-resistant enterococci were the *para*-substituted benzyl derivatives [e.g., *p*-phenyl, *p*-(*p*-chlorophenyl), *p*-butylbenzyl]. The range of MSC for these derivatives was 0.25-2 μg/ml [67]. During investigations *in vivo* the highest activity with respect to sensitive bacteria was exhibited by N-*p*-(*p*-chlorophenyl)benzyl-LY264826 (LY333328). Its derivative is at present undergoing clinical trials.





A report recently appeared on the reductive alkylation of the deacyl derivative of the antibiotic A-40926 at the amino group of the carbohydrate residue of 2-amino-2-deoxyglucuronic acid [68]. Substituents analogous with those described for LY264826 were used. However, the activity of the obtained derivatives with respect to glycopeptide-resistant enterococci proved not very high, as in the case of the alkyl derivatives of vancomycin and eremomycin (MSC 8-128 μg/ml).

The other important direction in the chemical modification of dalbaheptides is the synthesis of derivatives at the carboxyl group of the peptide core. The most interesting were the amides of dalbaheptides, although the esters [69-71], hydrazides [72], and carboxypeptides [73] exhibited high activity both *in vitro* and *in vivo*. The amides of eremomycin, the activity of which *in vivo* is comparable with the activity of the initial antibiotic, unlike the latter did not exhibit histamine-releasing activity [74]. The polybasic amides of the aglycone of teikoplanin were active *in vitro* toward certain clinical strains of Gram-negative bacteria having natural resistance to dalbaheptides [75, 76].

The derivative of the antibiotic A-40926 (MDL 63246), which is the most active *in vivo* among the natural and semisynthetic dalbaheptides with respect to sensitive Gram-positive bacteria, is also an amide [77, 78]. It was obtained by the reduction of N-acyl-2-amino-2-deoxyglucuronic acid to the corresponding glucosamine followed by amidation of the carboxyl group of the peptide core. In effectiveness this derivative surpasses vancomycin and teikoplanin by 10-30 times. However, the main disadvantage of MDL 63246 is its relatively low activity *in vitro* toward glycopeptide-resistant enterococci (MSC 432 μg/ml), and this substantially restrains the further advancement of this derivative in clinical practice.

As was shown above, dalbaheptides are polyfunctional compounds, and this leads to the low selectivity of many reactions. For example, the reductive alkylation of LY264826 or eremomycin is possible at all three amine groups. Although the amino group of the amino sugar in the disaccharide branch is alkylated preferentially, this reaction leads to complex mixtures of products, the separation of which is only possible by preparative HPLC. On the other hand, the introduction of lipophilic substituents at the carbonyl group does not give such an effect as alkylation [79]. Therefore, the search for new directions in the selective chemical modification of dalbaheptides is a pressing problem. One such direction is the aminomethylation of the aromatic (resorcinol) ring of the seventh amino acid. A series of aminomethyl derivatives containing both lipophilic and hydrophilic substituents were synthesized by the action of various amines and formaldehyde on eremomycin [80] or the aglycone of teikoplanin [81]. The aminomethyl derivatives of eremomycin with primary and secondary amines, α-, β-, and ε-amino acids, amino alcohols, primary diamines of various lengths, and even with ammonia were obtained. Substitution only occurred in the resorcinol ring of the seventh amino acid. Among more than 100 obtained derivatives the most active toward sensitive and resistant bacteria were the decylaminomethyl derivatives of eremomycin and the aglycone of teikoplanin. The MSC of these derivatives with respect to a series of glycopeptide-resistant strains of enterococci amounts to 4 μg/ml. At the same time the *p*-phenylbenzylaminomethyl derivatives of eremomycin and the aglycone of teikoplanin were practically inactive toward glycopeptide-resistant enterococci.

Thus, analysis of the structure—activity relation shows that an essential condition for the display of activity by derivatives of dalbaheptides toward resistant enterococci is the presence of lipophilic substituents of specific nature and size.

The introduction of saturated or unsaturated substituents with a long chain C_{10}, C_{11} has approximately the same effect on the activity of the dalbaheptides. Here the point of introduction of such substituents does not have a significant effect on the activity toward the resistant enterococci. However, modification of the dalbaheptides at the amino group of the sugars or in the resorcinol ring of the seventh amino acid is much preferred for combining high activity toward sensitive and resistant bacteria.

Different relationships are observed during the introduction of *p*-substituted benzyl radicals. Only derivatives of the antibiotic LY264826 exhibit high activity toward resistant enterococci, while the point of introduction of these substituents is strictly determined by the amino group of the amino sugar of the disaccharide branch. It is interesting that derivatives of dalbaheptides both with *p*-substituted benzyl and with aliphatic substituents hardly bond at all with the model peptide AcLys(Ac)-D-Ala-D-Lac [82]. The main mechanism of antibacterial action of such derivatives toward resistant bacteria is probably reaction of the lipophilic residue with the cytoplasmatic membrane of the bacterial wall (Fig. 3d) [9]. The lipophilic residue is incorporated in the membrane, and the antibiotic is fixed to the surface of the constructed cell wall, preventing further synthesis of the peptidoglycan and leading to destruction of the bacteria. This mechanism is probably more specific for the *p*-substituted benzyl derivatives of LY264826 at the amino group. It is also possible that elements of the cell wall of the bacteria, on which such derivatives act, may differ from those for derivatives with lipophilic aliphatic substituents.

The mechanisms of the interaction of natural and semisynthetic dalbaheptides given in Fig. 3 relate only to *in vitro* conditions. These mechanisms are considerably more complex for the *in vivo* case, when the pharmacokinetic characteristics have a large effect on the activity of the derivatives. It is therefore only possible to assess the prospects of a derivative after receiving the complete data from *in vivo* trials.

Convincing data have not yet been obtained to confirm the effectiveness of the semisynthetic derivatives of dalbaheptides examined above against glycopeptide-resistant enterococci *in vivo*. Time will show whether or not these derivatives will solve the problem of resistance.

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