A New Type of Chemical Modification of Glycopeptides Antibiotics: Aminomethylated Derivatives of Eremomycin and Their Antibacterial Activity

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A series of derivatives of eremomycin aminomethylated at the 7d position of the resorcinol ring of the amino acid No. 7 was prepared by interaction of eremomycin with formaldehyde and various primary and secondary amines and ammonia. The most active compound obtained was 7d-decylaminomethyl derivative, whose minimal inhibitory concentrations for clinical isolates of staphylococci are 2~8 times lower than those of the parent antibiotic. 7d-Decylaminomethyl derivative was also active against vancomycin-resistant VanA enterococci (8 μg/ml) and *Neisseria gonorrhoeae* (16 μg/ml).

Glycopeptides antibiotics of dalbaheptide group (vancomycin and teicoplanin) have been extensively used in the treatment of infections caused by methicillin-resistant and coagulase-negative staphylococci (CNS) as well as in therapy of multi-resistant enterococcal infections. Vancomycin and teicoplanin represent the last line of defence in the treatment of these refractory pathogens. The recent discovery of resistance to glycopeptides in VanA enterococci poses a serious threat for the future[1]. Some strains of CNS have also reduced susceptibility to teicoplanin and, occasionally, to vancomycin. It implies an urgent need for new and more potent glycopeptides with improved activity against multi-resistant staphylococci and activity against highly glycopeptide-resistant VanA enterococci.

A way to these objectives is chemical modification of the natural glycopeptides. The structural complexity of these antibiotics limits the variety of possible types of chemical modifications. Recently it has been shown that *N*-alkyl (*N*-aralkyl) derivatives of some glycopeptides exhibit improved antibacterial properties if alkyl or aralkyl substituents of a definite size and lypophilicity are introduced[2,3]. Thus, a search is promising for new types of chemical modifications of glycopeptides antibiotics which would permit the introduction of alkyl or aralkyl substituents into positions of the antibiotic molecule so far unaccessible.

Here we report a new type of chemical modification of the glycopeptide antibiotic eremomycin (**I**)[4] by the aminomethylation method (Mannich reaction), which led to compounds with improved antibacterial properties (Fig. 1).

Fig. 1. Structure of eremomycin and its aminomethylated derivatives.

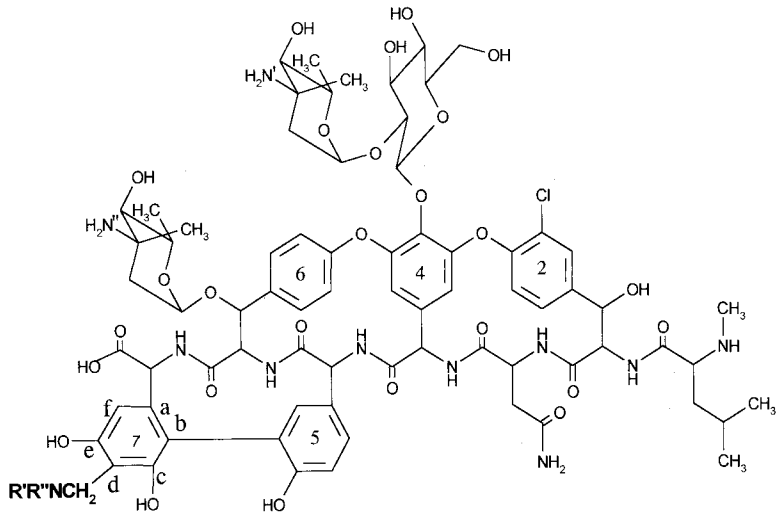


Table 1. Aminomethylated derivatives of eremomycin (**II**~**XVI**). Structures and properties.

Compound	NR′R″	Reaction time (hours)	Yield (%)	TLC Rf values Systems A	TLC Rf values Systems B	Electrophoretic mobility	Molecular formula	ESI-MS MW Calcd	ESI-MS MW Found
I	—	—	—	0.08	0.30	1.00	$C_{73}H_{89}N_{10}O_{26}Cl$	1556.3	—
II	NH_2	2	40	0.10	0.33	1.20	$C_{74}H_{92}N_{11}O_{26}Cl$	1585.3	1585.3
III	NHC_7H_{15}	18	48	0.40	0.60	1.12	$C_{81}H_{106}N_{11}O_{26}Cl$	1683.4	1683.6
IV	NHC_9H_{19}	18	45	0.49	0.68	1.10	$C_{83}H_{110}N_{11}O_{26}Cl$	1711.4	1711.5
V	$NHC_{10}H_{21}$	18	49	0.52	0.70	1.10	$C_{84}H_{112}N_{11}O_{26}Cl$	1725.4	1725.5
VI	$NHC_{12}H_{25}$	18	48	0.56	0.75	1.10	$C_{86}H_{116}N_{11}O_{26}Cl$	1735.4	1735.5
VII	$NHC_{18}H_{37}$	18	42	0.72	0.92	1.05	$C_{92}H_{128}N_{11}O_{26}Cl$	1837.5	1837.6
VIII	$N(CH_3)CH_2C_6H_4C_6H_5$-*p*	8	50	0.57	0.75	1.08	$C_{88}H_{104}N_{11}O_{26}Cl$	1765.7	1765.3
IX	$N[CH_2CH_2]_2N$-$N{=}CHC_6H_4Cl$-*p*	8	50	0.60	0.79	1.10	$C_{85}H_{103}N_{13}O_{26}Cl_2$	1791.7	1791.7
X	$NHCH_2C_6H_5$	18	50	0.34	0.52	1.12	$C_{81}H_{98}N_{11}O_{26}Cl$	1675.6	1675.5
XI	$N[CH_2CH_2]_2O$	6	49	0.16	0.47	1.15	$C_{78}H_{98}N_{11}O_{27}Cl$	1655.6	1655.6
XII	$N[CH_2CH_2]_2NCH_3$	6	48	0.14	0.45	1.24	$C_{79}H_{101}N_{12}O_{26}Cl$	1668.7	1668.8
XIII	$N(CH_3)_2$	8	49	0.12	0.36	1.18	$C_{76}H_{96}N_{11}O_{26}Cl$	1613.6	1613.5
XIV	$NH(CH_2)_4NH_2$	8	40	0.00	0.10	1.28	$C_{78}H_{101}N_{12}O_{26}Cl$	1656.7	1656.6
XV	$NH(CH_2)_2COOH$	18	45	0.00	0.21	0.96	$C_{77}H_{96}N_{11}O_{28}Cl$	1657.6	1657.6
XVI	$N(CH_3)CH_2(CHOH)_4$–CH_2OH	8	48	0.00	0.18	1.21	$C_{81}H_{106}N_{11}O_{31}Cl$	1763.7	1763.7

Chemistry

Aminomethylated derivatives of eremomycin (**II**~**XVI**) (Fig. 1) were prepared by the treatment of **I** with an excess of an amine in water or an acetonitrile - water 1 : 1 mixture, followed by the addition of aqueous formaldehyde in a five-fold molecular excess. Other organic solvent-water mixtures can be also used in this reaction. It is important to adjust pH of the reaction mixture to 9.5~10. Duration of the reaction depended on the type of amine. For example, when NH_3 was used, duration of the reaction was limited with 2 hours to prevent a side reaction of polymerisation. Aminomethylation of **I** proceeds with various amino components, including primary amines, diamines, amino acids and even ammonia. The reaction does not require preliminary protection of the amino groups and is directed exclusively to the 7d-position of the resorcinol ring of the amino acid No. 7 to yield the 7d-aminomethyl compounds in 40~50% yields. All the derivatives were purified by column chromatography on CM-32 cellulose followed by desalting with Amberlite XAD-2. The yields and properties of the compounds obtained are presented in Table 1.

The aminomethylated derivatives **II**~**XVI** were structurally elucidated with the use of the ^{1}H NMR and ESI-MS methods. The modification in the eremomycin molecule was located in the peptide moiety also by acid hydrolisis and Edman's degradation as previously described[5].

In ^{1}H NMR spectra all signals of the eremomycin backbone protons and the introduced substituents were identified. The most diagnostic were proton signals of the substituted resorcinol cycle. The doublet of 7d proton, which is present in ^{1}H NMR of **I**, was absent from ^{1}H NMR spectra of the aminomethylated derivatives **II**~**XVI**. In the ^{1}H NMR of eremomycin 7f proton is presented by a the doublet at 6.56 ppm, in ^{1}H NMR spectra of **II**~**XVI** the 7f proton signal had a form of a singlet. Cross-peaks in HMBC spectra between CH_2NR′R″ protons and 7e and 7c C-atoms of the resorcinol cycle unambigously revealed the position 7d for the introduced aminomethyl substituents. In addition, cross-peaks in ROESY spectra between CH_2NR′R″ protons and 7c-OH were observed.

Results and Discussion

Table 2 presents the *in vitro* antimicrobial activities of the aminomethylated derivatives of eremomycin (**II**~**XVI**) in comparison with those of the parent antibiotic. Most of the derivatives were at least as active as **I** against *S. haemolyticus* (clinical isolate) and streptococci. In general they had activity similar or even better than I against *S. epidermidis* (clinical isolate). But the majority of the aminomethylated derivatives was less active than **I** against *S. aureus*, especially clinical isolates of *S. aureus*. 7d-Decylaminomethyl derivative (**V**) was the most active compound, which had higher (2~8-fold) activities

Table 2. Antibacterial activity *in vitro* (μg/ml) of the aminomethylated derivatives of eremomycin (**II**~**XVI**) in comparison with **I**.

Compound	*Staphylococcus aureus* Smith L819	*S. aureus* L561 clinical isolate	*S. epidermidis* L533 clinical isolate	*S. haemolyticus* L602 clinical isolate	*Streptococcus pyogenes* C 203	*S. pneumoniae* UC 41	VanA Enterococci*
I	0.13	0.5	1	0.25	0.13	0.13	>128
II	0.5	4	0.5	0.5	0.13	0.25	>128
III	0.25	1	0.25	0.13	0.13	0.13	>128
IV	0.25	1	0.25	0.5	0.13	0.13	8~64
V	0.13	0.25	0.13	0.13	0.13	0.06	8
VI	0.25	1	0.5	0.5	0.25	0.13	16~32
VII	1	4	1	1	0.13	0.13	>128
VIII	0.5	32	8	0.5	0.13	0.13	32~128
IX	0.5	16	2	0.25	0.13	0.13	>128
X	0.5	2	0.25	0.25	0.13	0.13	>128
XI	1	8	1	0.5	0.13	0.13	>128
XII	1	4	0.5	0.25	0.25	0.13	>128
XIII	0.5	2	0.25	0.25	0.13	0.25	>128
XIV	0.13	1	0.25	0.13	0.06	0.25	>128
XV	4	32	2	2	1	0.5	>128
XVI	2	8	0.5	0.5	0.25	0.25	>128

* Range of MIC's for vancomycin-resistant (VanA) enterococci consisted of three strains: *E. faecalis* L562, *E. faecalis* L560, *E. faecium* L569.

Fig. 2. Relationships between the length of alkyl substituents of alkylaminomethyl derivatives (R′,R″-NCH_2-eremomycins) and activity *in vitro* against *S. aureus* clinical isolates.

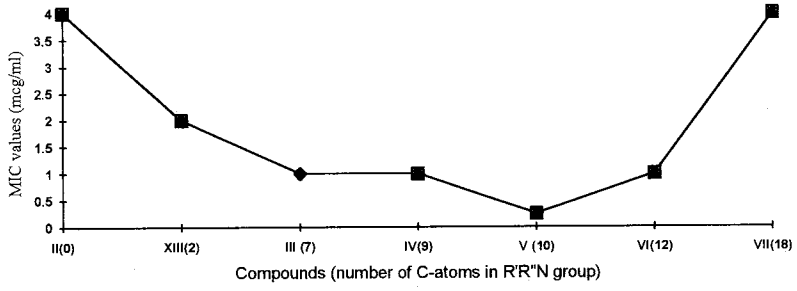
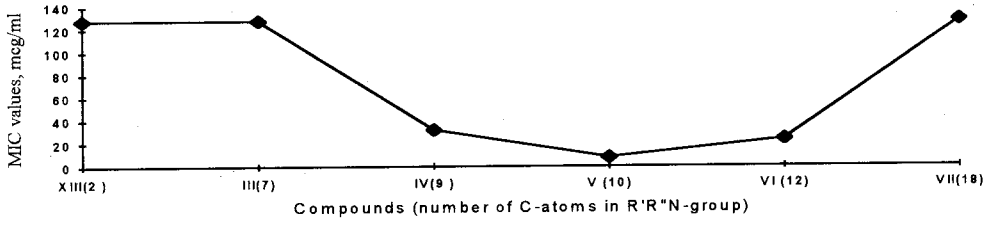


Fig. 3. Relationships between the length of alkyl substituents of alkylaminomethyl derivatives (R′R″-NCH_2-eremomycins) and activity *in vitro* against VanA enterococci (medium values for three strains: *E. faecalis* L562, *E. faecalis* L560, *E. faecium* L569).



against all Gram-positive bacteria studied than the parent antibiotic. Besides, **V** showed unexpected activity (16 μg/ml) against Gram-negative *Neisseria gonorrhoeae* ISM 68/126, while all other tested compounds were inactive against this strain. Activity of **V** against VanA enterococci is the most important. MIC values of **V** for three strains of VanA enterococci (two *E. faecalis* and one *E. faecium*) were 8 μg/ml, while MIC's of **I** for these strains were ≥256 μg/ml. Mannich derivatives **IV**, **VI** and **VIII** had marginal activity against VanA enterococci.

Earlier it was shown that among the *N′*-acyl- and *N′*-alkyl-eremomycins substituted in the eremosamine moiety of the disaccharide branch, C_{10} acyl and C_{10} alkyl analogues were the most active against Gram-

positive bacteria, including VanA enterococci[2,3]. MIC values of *N'*-decyleremomycin for VanA enterococci were 4~8 μg/ml. In our case similar regularities were observed for aliphatic aminomethylated derivatives, where C_{10} derivatives were the most active. High antibacterial activity was earlier found also for *N'*-(*para*-substituted benzyl or *p*-phenylbenzyl) derivatives (MIC values for VanA enterococci: 8~16 μg/ml). However, 7d-[*N*-(*p*-phenylbenzyl)-*N*-methylaminomethyl] eremomycin (**VIII**) had poor activity against both staphylococci and VanA enterococci.

The relationships between the length of the alkyl substituent in alkylaminomethyl derivatives and *in vitro* activity against *Staphylococcus aureus* and VanA enterococci are well seen in Fig. 2 and 3. Both decrease and increase of the chain length in comparison with the decylaminomethyl derivative lead to reduction of antibacterial activity.

Experimental

All reagents and solvents used were commercial products. The amine for synthesis of **VIII** was obtained by reductive alkylation of methylamine with *p*-phenylbenzaldehyde and $NaBH_4$ in MeOH. The hydrazone for the synthesis of **IX** was prepared from 1-aminopiperazine and *p*-chlorobenzaldehyde in MeOH. TLC was performed on the precoated Silica Gel $60F_{254}$ Merck plates in systems EtOAc - PrOH - 25% NH_4OH 2 : 1 : 1 (A) and 1 : 1 : 1 (B). Paper electrophoresis was performed in 0.05 M AcOH-pyridine buffer (pH 5.6) at 900 V for 3 hours using Filtrak F*N*-12 paper (Germany). CM-cellulose column chromatography was performed with LKB Ultragrad Gradient Mixer 11300 supplied with Uvicord 2138 (254 nm) and Recorder 2065. NMR spectra were recorded on a Varian VXR-400 spectrometer in D_2O at 70°C or CD_3OD at 40°C. ESI-MS spectra were obtained on Finnigan TSQ 700 instrument equipped with a Finnigan Electrospray ion source using the conditions described earlier[6].

Preparation of the Aminomethylated Derivatives of Eremomycin. General Procedure

To a stirred solution of 0.1 mmol of **I** and 0.6 mmol of an appropriate amine in ~10 ml of water or an acetonitrile - water 1 : 1 mixture, was added 0.5 mmol of 37% aqueous formaldehyde. If a salt of the amine was used, 1 N NaOH was added to pH 10. The reaction mixture was stirred at room temperature for 2~18 hours and then adjusted to ~pH 4 with 6 N H_2SO_4. Adding acetone (~60 ml) led to a precipitate, which was collected and dried in vacuum at room temperature for 4 hours. Then it was dissolved in a minimal amount of a 0.2 M CH_3COONH_4 - EtOH 4 : 1 mixture (pH 6.7) and applied to a chromatographic column with CM-32-cellulose (1 × 45 cm) preequilibrated with the same mixture. The column was developed with a linear gradient elution of NH_4OH (pH 6.7→9.8) in a 0.2 M CH_3COONH_4–EtOH 4 : 1 mixture at a rate of 0.5 ml/minute, while collecting 10 ml fractions. The suitable fractions were combined, acidified with 6 N H_2SO_4 to pH 7 and desalted for 24 hours with XAD-2 (~50 ml); (elution with 150 ml of a water - MeOH 1 : 1 mixture). The eluate was concentrated in a vacuum at 45°C to a small volume (~2 ml). After adding acetone (~60 ml) the precipitate formed was collected, rinsed with acetone and dried in vacuum at room temperature to give the pure aminomethyl compound.

Example 1: Preparation of 7d-Aminomethyleremomycin (II)

A stirred solution of 165 mg (0.1 mmol) of eremomycin sulfate and 52 mg (0.6 mmol) of NH_4Cl in 10 ml of water was adjusted to pH 10 with 1 N NaOH, and 0.038 ml (0.5 mmol) of 37% aqueous formaldehyde was added. The reaction mixture was stirred at room temperature for 2 hours and then adjusted to pH 4 with 6 N H_2SO_4. Adding acetone (60 ml) led to a precipitate, which was collected, dried in vacuum at room temperature, dissolved in 3 ml of a 0.2 M CH_3COONH_4 - EtOH 4 : 1 mixture (pH 6.7) and purified as described above to give 70 mg (40%) of **II**.

Example 2: Preparation of 7d-Decylaminomethyleremomycin (V)

To a stirred solution of 165 mg (0.1 mmol) of eremomycin sulfate and 0.12 ml (0.6 mmol) of decylamine in 12 ml of an acetonitrile - water 1 : 1 mixture, 0.038 ml (0.5 mmol) of 37% aqueous formaldehyde was added. The reaction mixture was stirred at room temperature for 18 hours, adjusted to pH 4 with 6 N H_2SO_4 and acetone (70 ml) was added, what led to a precipitate, which was collected, rinsed with acetone and dried in vacuum at room temperature. The purification was performed as described above to give 90 mg (50%) of **V**.

Determination of Antibacterial Activity *In Vitro*

MIC's were determined by broth microdilution assay in Difco Todd-Hewitt broth (streptococci), Oxoid Iso-Sensitest broth (staphylococci and enterococci) and

Difco GC Base broth +1% (v/v) IsoVitaleX (BBL) (*N. gonorrhoeae*). The inoculum was $\sim 10^5$ cfu/ml. Incubation was for 24 hours at 37°C. For *N. gonorrhoeae* incubation was for 48 hours in CO_2-enriched atmosphere.

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