New Semisynthetic Glycopeptides MDL 63,246 and MDL 63,042, and Other Amide Derivatives of Antibiotic A-40,926 Active against Highly Glycopeptide-resistant VanA Enterococci

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A series of amide derivatives of natural glycopeptide A-40,926 (A), its 6^B-methyl ester (MA) and 6^B-decarboxy-6^B-hydroxymethyl derivative (RA) were prepared with the aim of obtaining activity against glycopeptide-resistant enterococci.

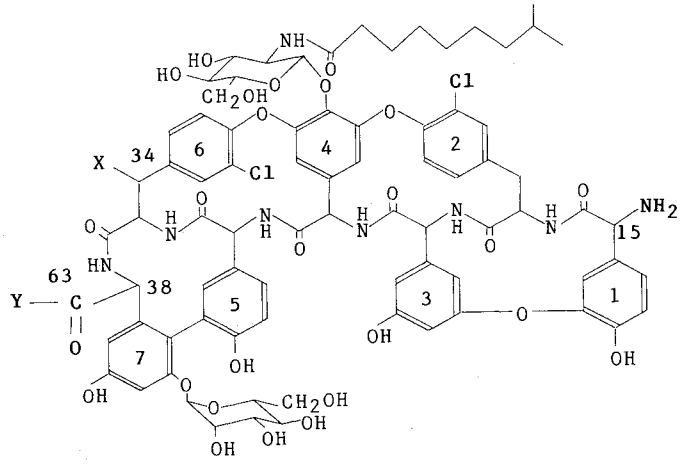
These compounds are structurally related to a class of amides of 34-de(acetylglucosaminyl)-34-deoxy teicoplanin which showed interesting activity against strains of *Enterococcus faecalis* and *E. faecium* highly resistant to both vancomycin and teicoplanin. Among them, RA-amides MDL 63,246 and MDL 63,042 were the most active derivatives against several Gram-positive bacteria, including VanB and VanC enterococci, and were moderately active (MIC range $0.5 \sim 64\ \mu g/ml$) against strains of *Enterococcus* for which vancomycin and teicoplanin MICs were $\geq 128\ \mu g/ml$.

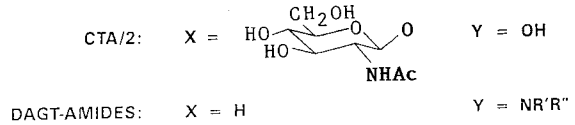
The chemical rationale and the synthesis of these new series of glycopeptide derivatives are described. Preliminary *in vitro* data are reported and structure-activity relationships are discussed.

In the last few years an increase in serious infections caused by enterococci has been observed in hospitalized patients. Clinical isolates of enterococci are intrinsically resistant to many classes of antibacterial drugs, and infections often require treatment with a combination of agents to which they are moderately susceptible. The recent appearance of vancomycin-resistant enterococci poses a serious threat for the near future, particularly because high-level resistance is often associated with genetic elements which can spread from one bacterial strain to another. The emerging resistance in enterococci[1] is a current challenge for glycopeptides of the dalbaheptide[2] family since teicoplanin (Fig. 1),[3] the only other glycopeptide in clinical use, is active against some but not all vancomycin-resistant strains. Semisynthetic glycopeptides MDL 63,246 and MDL 63,042 (Fig. 2) are the result of a new chemical program of transformation of teicoplanin and teicoplanin-like antibiotics aimed at broadening their activity to highly glycopeptide-resistant enterococci.

Among the teicoplanin derivatives, some basic amides (Fig. 1)[4] of the 34-de(acetylglucosaminyl)-34-deoxy pseudoaglycone had interesting *in vitro* activity against strains of *Enterococcus faecalis* and *E. faecium* highly

Fig. 1. Structures of teicoplanin A2-2 (CTA/2) and of the amides (DAGT-AMIDES) of its 34-de(acetylglucosaminyl)-34-deoxy pseudoaglycone.





resistant to both vancomycin and teicoplanin. These acetylglucosamine-less derivatives also differ from corresponding teicoplanin amides in the absence of the oxygen at C-34. The benzylic hydroxyl group on amino acid 6 is always present in natural dalbaheptides and seems to contribute to stabilization of the macrocyclic ring formed by amino acid fragments 5, 6 and 7 in the natural conformation in which the 5,6-peptide bond is "*cisoid*".

The teicoplanin-like antibiotic A-40,926 (Fig. 3),[5,6] was considered to be a more suitable starting material than teicoplanin for the preparation of derivatives containing the original 34-hydroxyl group, as it lacks the 34-acetylglucosamine. It has a mannose and a single *N*-acylamino sugar in the same positions as teicoplanin. To further increase the similarities between these

Fig. 2. Structures of MDL 63,246 (RA-A-1) and MDL 63,042 (RA-A-2) (with proton nomenclature).

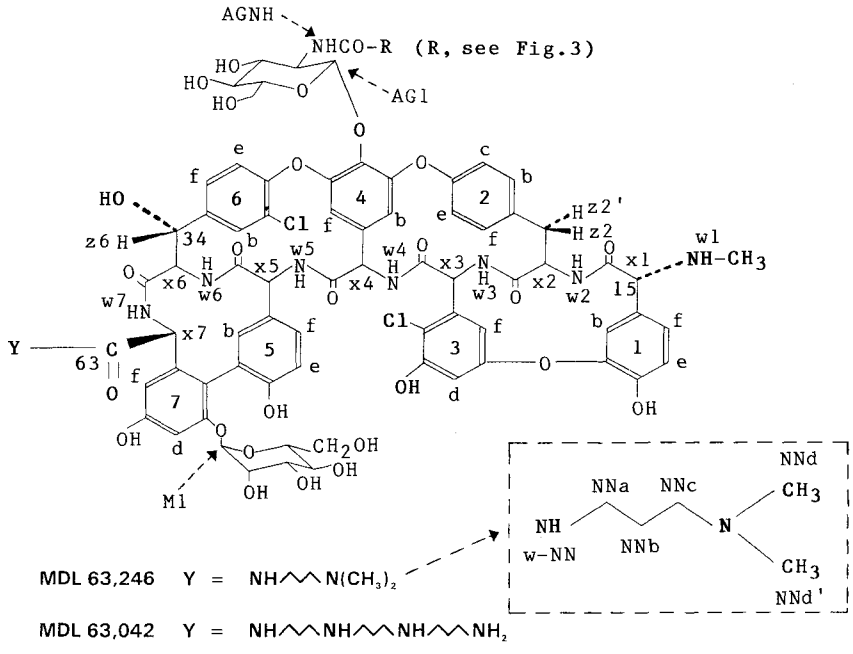
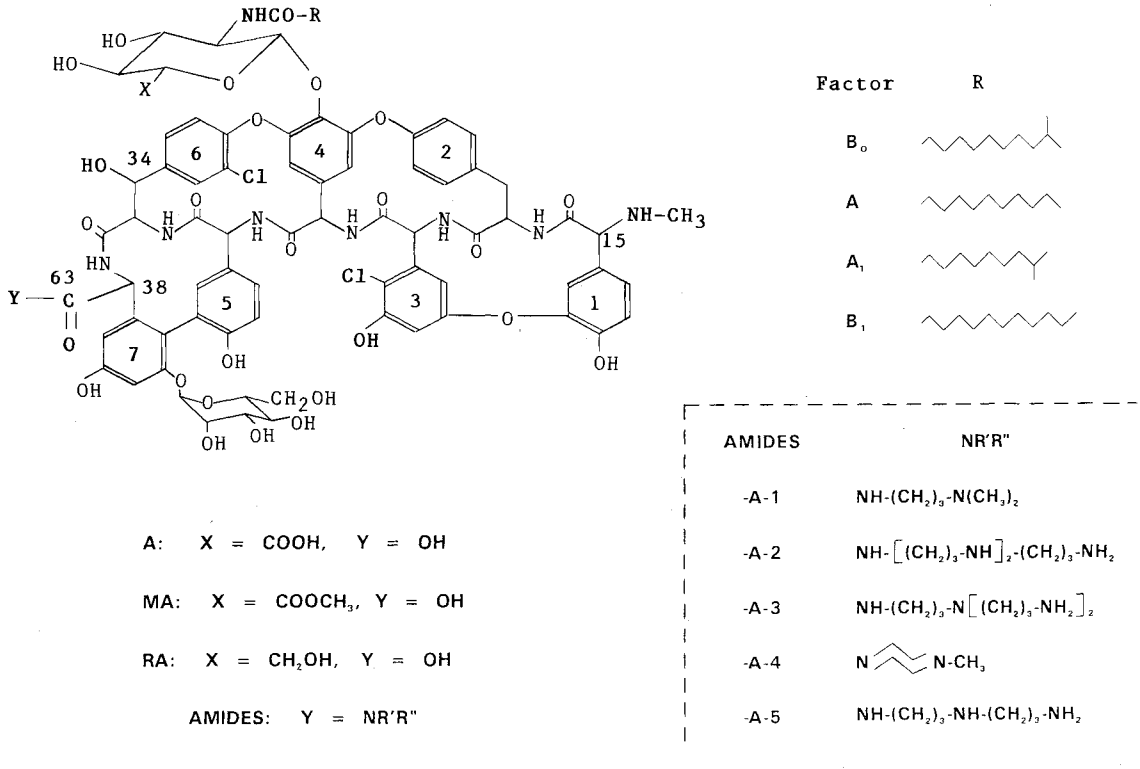


Fig. 3. Structures of A (A-40,926), MA, RA and of their amide derivatives.



molecules, the sugar-carboxyl group of A-40,926 was reduced to alcohol.

In this paper the synthesis and preliminary *in vitro* antibacterial activities of a series of N^{63}-carboxamides of A-40,926 (A), its 6^{B}-methyl ester (MA) and 6^{B}-decarboxy-6^{B}-hydroxymethyl derivative (RA) are described, with particular reference to the RA-amides MDL 63,246 (RA-A-1) and MDL 63,042 (RA-A-2).

To assess the influence of mannose on the activity of MDL 63,246 and its synthetic precursors, demannosyl (DM) derivatives DMA, DMMA, DMRA (Fig. 4), and their amides (DMA-A-1, DMMA-A-1, DMRA-A-1) with 3,3-dimethylamino-1-propylamine were also prepared. Finally, the sugar-free compound DMGA-A-1 (the aglycone of MDL 63,246)[†1] was synthesized to establish the role of the *N*-acylglucosamine on the activity of these compounds against VanA enterococci.

Chemistry

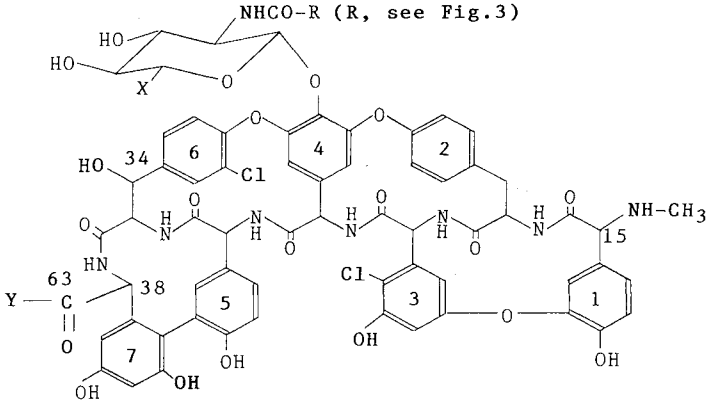
The reduction of the *N*-acylglucuronic acid of A to obtain the *N*-acylglucosaminyl derivative RA was carried out *via* the mono-methyl ester MA intermediate, according to the procedure (Method A) outlined in Scheme 1a.

The selective esterification of the sugar-carboxyl group of A was performed in methanolic solution at room temperature in the presence of a catalytic amount of H_2SO_4. The *N*-terminal methylamino group of the resulting ester (MA) was suitably protected as *tert*-butyl (BOC) carbamate to prevent the possible reductive-hydrolysis of the 2,3-amide bond[7] with $NaBH_4$. Reduction of the sugar-methyl ester of the N^{15}-BOC-protected MA (BOC-MA) to alcohol was obtained upon treatment with an excess of $NaBH_4$ in a H_2O-BuOH (2:1) mixture at room temperature, yielding the BOC-derivative (BOC-RA) of RA. The final deprotection step was performed under controlled acidic conditions, in trifluoroacetic acid (TFA) at 0°C.[†2]

The N^{63}-carboxamide derivatives (MA-A-1~5) of MA and (RA-A-1~5) of RA (Fig. 3, Table 1) were prepared by condensation of the C-38-carboxyl group of MA and RA, respectively, with selected amines in dimethylsulfoxide (DMSO) at room temperature in the presence of benzotriazolyloxy-*tris*-pyrrolidinophosphonium-hexafluoro-phosphate (PyBOP), as the condensing agent (Scheme 2a). The amides (A-A-1~3) of A were obtained by saponification of the sugar-methyl ester of the corresponding MA-amides with 1 N NaOH at room temperature in a THF-H_2O (6:1) mixture (Scheme 2b).

Selective demannosylation of A or MA, to give DMA or DMMA (Fig. 4, Table 2) was achieved under acidic conditions (37% HCl in DMSO, at 55~60°C for 15

Fig. 4. Structures of demannosyl compounds DMA, DMMA, DMRA, and of their 3,3-dimethylamino-1-propylamide derivatives DMA-, DMMA- and DMRA-A-1.

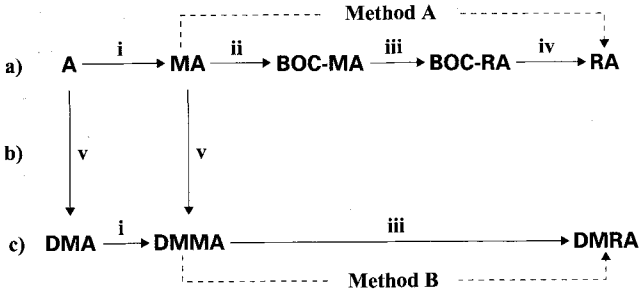


DMA: X = COOH, Y = OH — DMA-A-1,
DMMA: X = $COOCH_3$, Y = OH — DMMA-A-1,
DMRA: X = CH_2OH, Y = OH — DMRA-A-1
Y = NH~N

[†1] DMGA-A-1 is the aglycone moiety common to RA-A-1, A-A-1 and MA-A-1 (Fig. 3).

[†2] In contrast to the acylglucuronic moiety and its methyl ester present in A and MA, respectively, which are stable under relatively strong acidic conditions, the hemiacetalic bond of the acylglucosamine of RA is susceptible to hydrolysis by TFA even at room temperature. The instability of this sugar in RA is comparable to that of the acylglucosamine in teicoplanine antibiotics.[3]

Scheme 1. Synthesis of MA, RA (Fig. 3), and demannosyl (DM) derivatives DMA, DMMA and DMRA (Fig. 4).



i. $MeOH/H_2SO_4$, ii. $BOC_2O/NaHCO_3$ (water-dioxane 1:1), iii. $NaBH_4$ (H_2O-BuOH 2:1), iv. TFA, 0°C, v. 37% HCl, 55~60°C (DMSO).

Scheme 2. Synthesis of the amides of A, MA and RA (Fig. 3, and of DMA-, DMMA- and DMRA-A-1 (Fig. 4) and their aglycone DMGA-A-1.

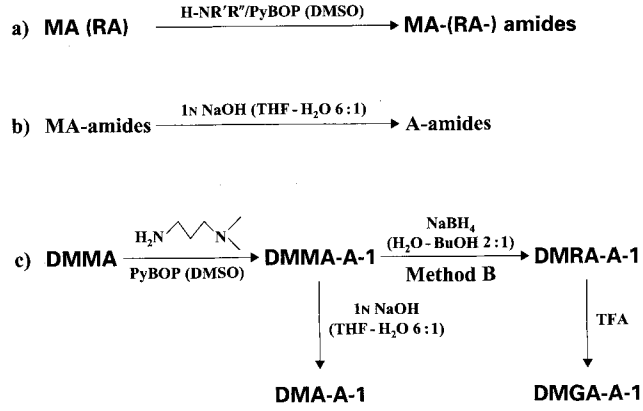


Table 1. Derivatives of A-40,926-6^B-methyl ester (MA), of 6^B-decarboxy-6^B-hydroxymethyl-A-40,926 (RA), and of A-40,926 (A): Fig. 3.

Compound	X	Y	Yield (%)	HPLC[a] t_R (minutes)	Formula[a]	MW[a] (calcd)
MA	$COOCH_3$	OH	97	11.3	$C_{84}H_{90}N_8O_{29}Cl_2$	1746.6
MA-A-1	$COOCH_3$	$NH(CH^2)_3N(CH_3)_2$	63	14.7	$C_{89}H_{102}N_{10}O_{28}Cl_2$	1830.8[b]
MA-A-2	$COOCH_3$	$NH[(CH_2)_3NH]_2(CH_2)_3NH_2$	51	15.5	$C_{93}H_{112}N_{12}O_{28}Cl_2$	1916.9
MA-A-3	$COOCH_3$	$NH(CH_2)_3N[(CH_2)_3NH_2]_2$	37	15.3	$C_{93}H_{112}N_{12}O_{28}Cl_2$	1916.9
MA-A-4	$COOCH_3$	$N[(CH_2)_2]_2NCH_3$	73	14.8	$C_{89}H_{100}N_{10}O_{28}Cl_2$	1828.7
MA-A-5	$COOCH_3$	$NH(CH_2)_3NH(CH_2)_3NH_2$	58	15.1	$C_{90}H_{105}N_{11}O_{28}Cl_2$	1859.8
RA	CH_2OH	OH	60	10.2	$C_{83}H_{90}N_8O_{28}Cl_2$	1718.6
RA-A-1 (MDL 63,246)	CH_2OH	$NH(CH_2)_3N(CH_3)_2$	70	13.5	$C_{88}H_{102}N_{10}O_{27}Cl_2$	1802.7[c]
RA-A-2 (MDL 63,042)	CH_2OH	$NH[(CH_2)_3NH]_2(CH_2)_3NH_2$	41	15.0	$C_{92}H_{112}N_{12}O_{27}Cl_2$	1888.9[d]
RA-A-3	CH_2OH	$NH(CH_2)_3N[(CH_2)_3NH_2]_2$	20	14.8	$C_{92}H_{112}N_{12}O_{27}Cl_2$	1888.9
RA-A-4	CH_2OH	$N[(CH_2)_2]_2NCH_3$	55	13.7	$C_{88}H_{100}N_{10}O_{27}Cl_2$	1800.7
RA-A-5	CH_2OH	$NH(CH_2)_3NH(CH_2)_3NH_2$	39	14.5	$C_{89}H_{105}N_{11}O_{27}Cl_2$	1831.8
A-A-1	COOH	$NH(CH_2)_3N(CH_3)_2$	41[e]	12.1	$C_{88}H_{100}N_{10}O_{28}Cl_2$	1816.7
A-A-2	COOH	$NH[(CH_2)_3NH]_2(CH_2)_3NH_2$	47[e]	12.4	$C_{92}H_{110}N_{12}O_{28}Cl_2$	1902.9
A-A-3	COOH	$NH(CH_2)_3N[(CH_2)_3NH_2]_2$	41[e]	12.2	$C_{92}H_{110}N_{12}O_{28}Cl_2$	1902.9
A	COOH	OH	—	9.7	$C_{83}H_{88}N_8O_{29}Cl_2$	1732.5

[a] Data for the main factor B_o.
[b] FAB MS: $[MH]^+ = 1829.6$.
[c] FAB MS: $[MH]^+ = 1801.6$.
[d] FAB MS: $[MH]^+ = 1887.7$.
[e] From the corresponding MA-amide.

Table 2. Demannosyl compounds (DM-derivatives, Fig. 4) and aglycone DMGA-A-1.

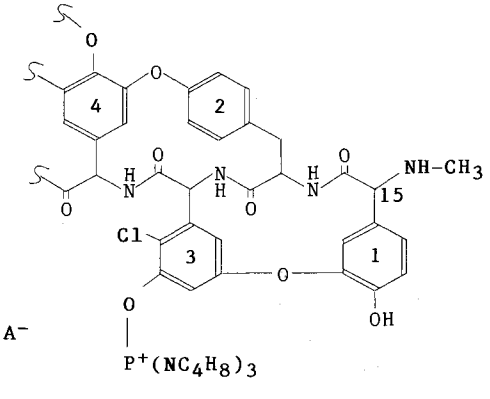
Compound	X	Y	Yield (%)	HPLC[a] t_R (minutes)	Formula[a]	MW[a] (calcd)
DMA	COOH	OH	23	10.1	$C_{77}H_{78}N_8O_{24}Cl_2$	1570.4
DMMA	$COOCH_3$	OH	29	12.7	$C_{78}H_{80}N_8O_{24}Cl_2$	1584.4
DMRA	CH_2OH	OH	15	10.9	$C_{77}H_{80}N_8O_{23}Cl_2$	1556.4
DMA-A-1	COOH	$NH(CH_2)_3N(CH_3)_2$	44	13.1	$C_{82}H_{90}N_{10}O_{23}Cl_2$	1654.6
DMMA-A-1	$COOCH_3$	$NH(CH_2)_3N(CH_3)_2$	47	17.9	$C_{83}H_{92}N_{10}O_{23}Cl_2$	1668.6
DMRA-A-1	CH_2OH	$NH(CH_2)_3N(CH_3)_2$	20	14.7	$C_{82}H_{92}N_{10}O_{22}Cl_2$	1640.6
DMGA-A-1	—[b]	$NH(CH_2)_3N(CH_3)_2$	35	5.9	$C_{64}H_{59}N_9O_{17}Cl_2$	1297.1

[a] Data for the main factor B_o.
[b] The acyl sugar on ring 4 is missing (Fig. 4).

hours) which did not affect their *N*-acyl sugar on amino acid 4 (Scheme 1b). The demannosyl derivative (DMRA) of RA was prepared (Scheme 1c) by reduction of the sugar-methyl ester of DMMA with $NaBH_4$ (H_2O-BuOH, 2:1, at room temperature) without protecting the *N*-teminal methylamino group (Method B). Condensation of the 63-COOH of DMMA with the 3,3-dimethylamino-1-propylamine was carried out (Scheme 2c) as described above in the synthesis of MA-amides. Reduction of the sugar-methyl ester of the resulting DMMA-A-1 with $NaBH_4$ under the usual conditions (Method B) gave DMRA-A-1. Removal of the *N*-acylglucosamine from the latter compound with TFA at room temperature gave the corresponding aglycone DMGA-A-1. Demannosylated amide DMA-A-1 was obtained from DMMA-A-1 according to the same procedure (1 N NaOH, THF-H_2O, 6:1, at room temperature) used in the preparation of A-amides from MA-amides.

The use of PyBOP as the condensing agent in the synthesis of MA- and RA-amides and DMMA-A-1 required well defined reaction conditions to minimize or prevent the formation of by-products (PyMA- and PyRA-amides) due to addition of the *tris*-pyrrolidinophosphonium moiety to the phenolic oxygen of aromatic ring 3 (Fig. 5). This side reaction, which is favored by basic conditions and by an excess of PyBOP, is due to the relatively strong acidity (pK_{MCS} 9.2) of the phenolic hydroxyl group of amino acid 3, attributable to the presence of a Cl atom in the *ortho* position.

Fig. 5. Structure of *tris*-pyrrolidinophosphonium (Py-) by-products.



Small samples of PyMA-A-1 and PyRA-A-1 (Table 3) were properly synthesized from MA and RA, respectively, using an excess of PyBOP and dimethylaminopropylamine, while PyA-A-1 was then obtained upon treatment of PyMA-A-1 with 1 N NaOH under the usual conditions (THF-H_2O 6:1, room temperature).

All derivatives described here are mixtures of four strictly related components, namely factors B_0 ($\geq 70\%$),[†3] and A, A_1 and B_1 (together, $\leq 25\%$), which differ only in the structure of the aliphatic chain of the *N*-acyl sugar on amino acid 4, plus an additional component, DMB_0 ($\leq 5\%$),[†4] which differs from B_0 in the absence of the mannose on aromatic ring 7. The structures of factors B_0, A, A_1 and B_1 (Fig. 3) and their percent composition were the same in all derivatives. The course of the reactions and the homogeneity[†5] of the final compounds were checked by HPLC.

Structure Elucidation

The structures of these products were determined by ^1H NMR spectroscopy and, in some cases, confirmed by FAB MS spectrometry and acid-base titration. The IR[†6] and UV[†7] spectra of this series of compounds were in accordance with the structures reported.

NMR Spectroscopy of RA-A-1

The structure of RA-A-1 (Fig. 2)[†8] was particularly investigated and confirmed by extensive NMR studies. The proton resonances have been assigned by homonuclear proton correlation spectroscopy using DQF-COSY,[8] TOCSY,[9] ROESY[10] and NOESY[11] experiments. A complete assignment of all carbon resonances was achieved by HMQC[12] and HMBC[13] spectra.

Proton chemical shifts: The TOCSY and COSY spectra reveal the presence of 7 amide protons belonging to the spin systems of 5 AX systems (w3, w4, w5, w6, w7), 1 AXYY′ system (w2) and 1 AXX′YY′ system (wNN). To distinguish between the 5 AX spin systems (w6/x6 appears as an AX system due to the weak coupling between x6 and z6) a sequential assignment using a ROESY or a NOESY experiment was necessary.

[†3] Expressed as percentage of the areas of peaks (HPLC).

[†4] Demannosyl (DM) derivatives are mixtures of four components: B_0 ($\geq 75\%$), and A, A_1 and B_1 ($\leq 25\%$).

[†5] Dealing with mixtures of five (four) components, HPLC analysis of final products was needed to assess the homogeneous composition of all derivatives in the five components and to check the absence of impurities or by-products.

[†6] The IR spectra were useful to confirm the presence of the methyl ester function (ν_{CO}, 1740 cm^{-1}) in MA-, DMA- and DMMA-derivatives.

[†7] The UV spectra of these derivatives are characterized by an absorption maximum at 281 nm.

[†8] The nomenclature of protons is that generally adopted for teicoplanin[3] and other dalbaheptides.

The starting point has been the amide proton wNN on the carboxyl terminus which was easily identified by its unique spin system. Some problems which occurred due to signal overlapping (x5/x7, w3/w4) were overcome by using additional cross relaxations between the amide protons and protons in the side chains (for example w7/z6 and w5/4f). This is also true for the connectivity between residue 3 and 2; the cross peak between w3 and x2 is very weak but w3 experiences a strong cross relaxation to z2'.

In the Hα region of the ROESY and NOESY spectrum of RA-A-1 two very strong cross peaks were obtained both including x6. One of them is due to the correlation with z6 which could not be assigned by scalar coupling. The second peak corresponds to cross relaxation either with x5 or x7 which have identical chemical shift values. However, in the spectrum of the aglycone of A-40,926[14] these two protons have different chemical shifts and therefore the ROE can be attributed unambiguously to x5 indicating a *cis*-bond between residue 5 and 6 which is characteristic for all glycopeptides of this family (Fig. 6).

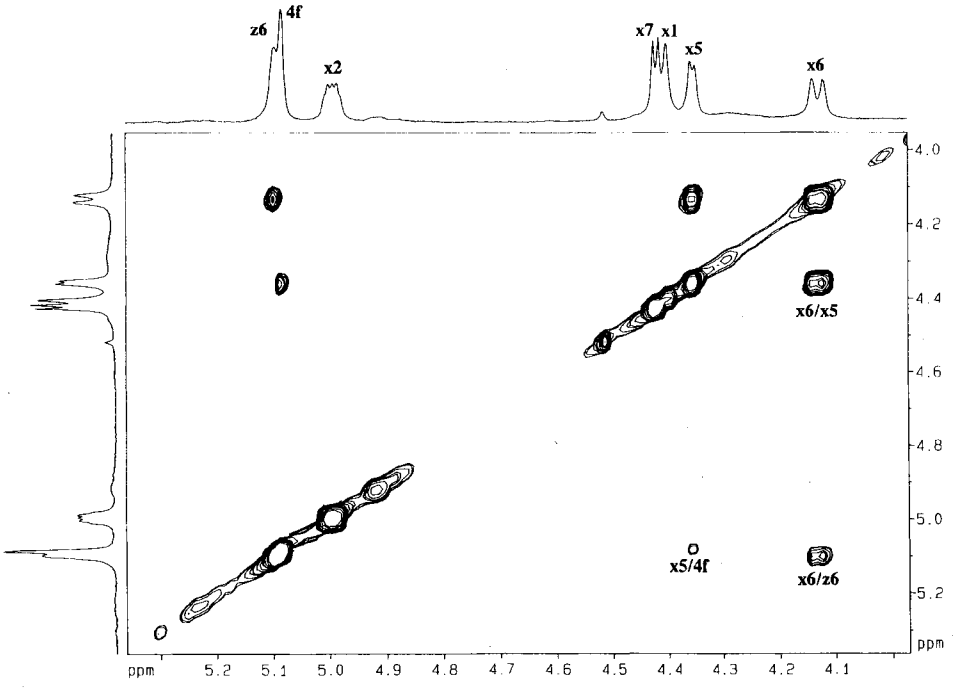
The TOCSY and COSY spectra were also used for the assignment of the 7 aromatic spin systems. The protons are either correlated with strong coupling constants (in the case of vicinal protons) or relatively weak coupling constants (1~2 Hz for long range correlations). All protons which belong to the same ring are connected *via* scalar coupling in the TOCSY spectrum. In this way it was possible to determine one 1,4-disubstitued (ring 2),

Table 3. *Tris*-Pyrrolidinophosphonium by-products (Py-derivatives, Fig. 5).

Compound	X[b]	Y[b]	Yield (%)	HPLC[a] t_R (minutes)	Formula[a]	MW[a] (calcd)
PyA-A-1	COO^-	$NH(CH_2)_3N(CH_3)_2$	35	18.5	$C_{100}H_{122}N_{13}O_{28}PCl_2 \cdot CH_3COOH$[c]	2116.1[d]
PyMA-A-1	$COOCH_3$	$NH(CH_2)_3N(CH_3)_2$	70	21.3	$C_{101}H_{125}N_{13}O_{28}PCl_2 \cdot CH_3COO^- \cdot CH_3COOH$[e]	2190.1[f]
PyRA-A-1	CH_2OH	$NH(CH_2)_3N(CH_3)_2$	47	19.3	$C_{100}H_{125}N_{13}O_{27}PCl_2 \cdot CH_3COO^- \cdot CH_3COOH$[e]	2162.1[g]

[a] Data for the main factor B_0.
[b] X and Y, as in Fig. 3 for corresponding amides A-, MA- and RA-A-1.
[c] Internal salt [sugar-COO^- $^+P(NC_4H_8)_3$], mono-acetate AcO^- $^+HN(CH_3)_2$
OPh $(CH_2)_3$–NH–CO-----
[d] MW: 2056.013 (internal salt) + 60.054 (AcOH).
[e] Di-acetate salt: [AcO^- $^+P(N(C_4H_8)_3]$, AcO^- $^+HN(CH_3)_2$
OPh $(CH_2)_3$–NH–CO-----
[f] MW: 2130.090 (2071.048 + 59.042, phosphonium acetate) + 60.054 (AcOH).
[g] MW: 2102.079 (2043.037 + 59.042, phosphonium acetate) + 60.054 (AcOH).

Fig. 6. Hα Region of the ROESY spectrum of the aglycone of A-40,926.



three 1,2,4-trisubstituted (rings 1, 5 and 6) and three 1,2,3,5 tetrasubstituted rings (rings 3, 4 and 7). The correct assignment of the aromatic rings was carried out by ROEs between the aromatic protons and protons in the peptide backbone.

The resonances of the two sugar moieties were assigned by COSY, TOCSY and ROESY experiments using the anomeric protons as starting points. The anomeric protons were easily identified by the chemical shifts of the corresponding carbon atoms in the HMQC spectrum. The anomeric proton (AG1) of the glucosamine unit shows strong scalar coupling to the proton in position 2 and appears therefore as a doublet (β-glycoside linkage) whereas the anomeric proton (M1) of the mannose unit shows a very small coupling to the proton in position 2 due to the equatorial position of M1 (α-glycoside linkage). Because of exchange broadening it was not possible to assign the hydroxyl resonances.

Starting points for the assignment of the aliphatic chain of the fatty acid moiety of factor B_0 were the two terminal methyl groups at 0.85 ppm and the αCH_2 group directly connected to the acyl-carbonyl carbon. The coupling pattern in the COSY spectrum allowed the assignment of the α and β CH_2s and of the CH proton of the terminal isopropyl moiety; the other protons appear at very similar chemical shifts between 1.2 and 1.0 ppm and could not be distinguished.

Carbon chemical shifts: The chemical shifts of all carbons which are directly connected to a proton were assigned by a HMQC spectrum; for all quaternary carbons a HMBC spectrum was required.

The carbonyl carbons were identified by their long range couplings to the amide protons (w2~w8) and/or the α-protons (x1~x7). The carbonyl function of the fatty acid chain is correlated with the αCH_2 protons and the amide proton (AG-NH) of the glucosamine. In some cases the cross peaks between the amide protons and the carbonyl carbons appear very weak due to line broadening of the proton resonances. However, the assignment is in accordance with the results obtained for the aglycone of A-40,926 where less line broadening is observed.

The aromatic carbons to which no protons are attached can be assigned by long range correlation to the aromatic protons on neighbouring carbons. Usually only the

Fig. 7. Region of the ^1H NMR spectrum of RA-A-1 at different temperatures.

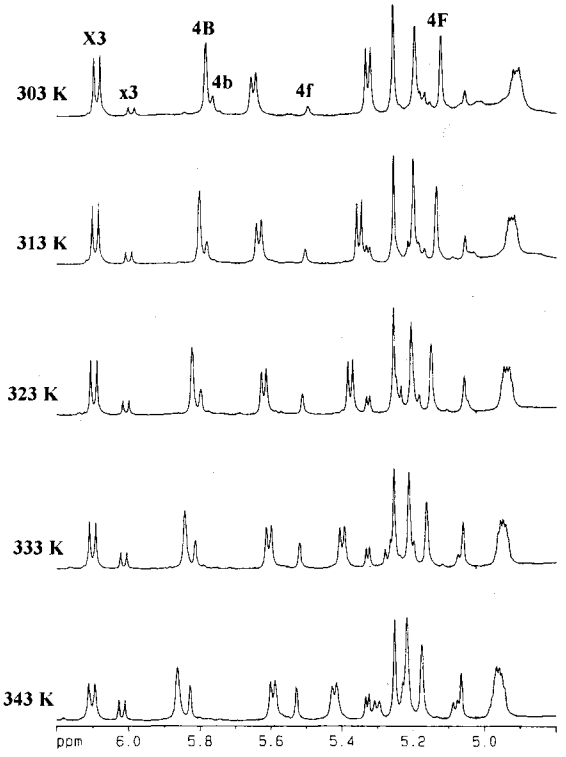
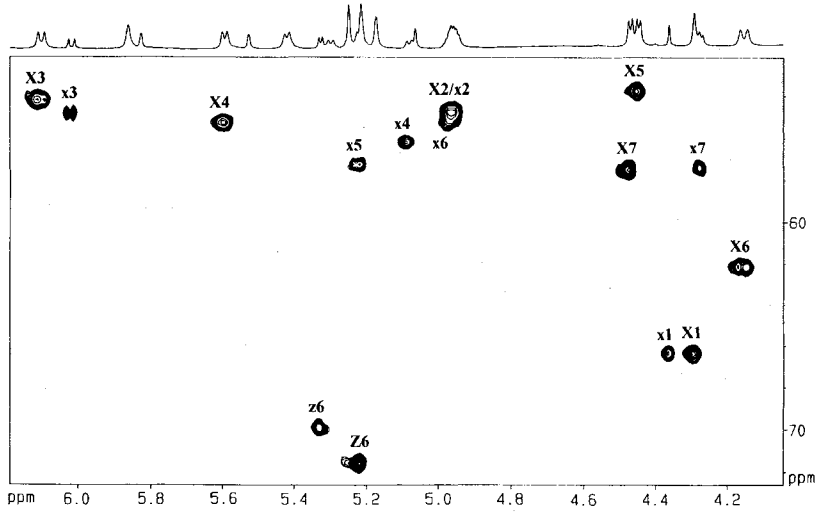


Fig. 8. α-Region of the HMQC spectrum of RA-A-1 at 70°C.



strong 3-J(C,H) couplings are observed. The 2-J(C,H) coupling constants are normally in the order of 1~2 Hz and therefore not detectable in the HMBC spectrum. However, if the carbon is carrying an electronegative group (OH, OR, Cl) the 2-J(C,H) becomes 3~4 Hz and can be observed.[15]

In DMSO, RA-A-1 exists in two unequally populated conformations which are in slow exchange on the NMR time scale and give rise to two sets of signals in the NMR spectrum. A similar behaviour has been previously described for the structurally related glycopeptide teicoplanin.[16] As shown in Fig. 7, the ratio of the two conformers is strongly temperature dependant. The portion of the minor conformation increases from 12% at room temperature to 23% at 70°C. The two conformations do not exchange fast enough, even at 70°C, to observe exchange NOEs in the ROESY or the NOESY spectra. These exchange NOEs are usually used to assign easily the resonances of a less populated conformation when other problems arise due to weak signal intensities and/or signal overlapping. In the case of RA-A-1, the assignment of the minor conformation must be carried out independently from the attribution of the major one.

To obtain a better signal to noise ratio a TOCSY and a HMQC experiment were performed at 70°C, a temperature at which the portion of the minor conformation is significantly increased (Fig. 8). Using these data in combination with the spectra recorded at 30°C it was possible to assign all protons of the peptide core and the anomeric sugar protons. Table 4 gives the proton and carbon chemical shifts of the minor conformation at 30°C in comparison with the corresponding values of the major one. It can be seen that most of the resonances differ significantly indicating a conformational change of the overall structure of the peptide ring.

One of the structural characteristics of this family of glycopeptides is the presence of a *cis* peptide bond between residues 5 and 6 which is reflected by a very intense NOE between the two Hα-protons (x5 and x6). In the case of the minor conformation such a NOE is not observed which suggests a trans configuration between these residues. The relative rigidity of the peptide core could be the origin of a high energy barrier between the two conformers and therefore be responsible for the slow exchange.

The existance of two different configurations between residue 5 and 6 is supported by the fact that the most striking changes in proton and carbon chemical shifts occur in this region. This is true for the backbone as well as for the side chains (see aromatic protons of ring 5 and 6). Another obvious difference is present in the signal of z6 which appears as a doublet in the minor conformation and as a singlet in the major one showing a different coupling and therefore a different dihedral angle between z6 and x6.

Table 4. Chemical Shifts (δ, ppm) of the ^1H and ^{13}C NMR spectra of RA-A-1 (Fig. 2).

Assignment	Major conformation		Minor conformation	
	^1H	^{13}C	^1H	^{13}C
1-CH_3	2.33	34.1	2.35	34.1
x1	4.31	66.1	4.38	65.9
1b	6.60	115.9	6.90	118.4
1e	6.89	117.5	6.95	117.7
1f	7.04	124.7	7.10	125.3
w2	7.47	—	7.60	—
x2	4.91	54.4	4.91	54.4
z2	3.33/2.81	37.4	3.29/2.86	37.4
2b	7.10	130.6	6.98	131.3
2c	7.18	124.1	6.97	122.7
2e	7.09	122.6	7.24	121.5
2f	7.62	131.1	7.63	130.0
w3	7.63	—	7.77	—
x3	6.08	53.8	5.99	54.6
3d	6.69	107.4	6.69	106.0
3f	6.48	107.1	6.70	105.0
w4	7.64	—	7.18	—
x4	5.65	54.9	5.01	56.0
4b	5.78	108.3	5.77	107.3
4f	5.12	103.9	5.50	104.5
w5	8.50	—	9.20	—
x5	4.42	53.6	5.16	57.1
5b	7.10	135.3	6.54	131.0
5e	6.71	116.1	6.76	114.3
5f	6.71	125.8	6.52	123.1
w6	6.69	—	broad	—
x6	4.13	62.1	4.89	55.2
z6	5.19	71.5	5.32	69.7
6b	7.73	127.1	7.17	127.8
6e	7.30	123.4	7.34	124.5
6f	7.43	126.9	7.60	125.8
w7	8.39	—	9.10	—
x7	4.41	57.3	4.20	57.2
7d	6.71	101.0	6.65	102.3
7f	6.42	108.0	6.40	108.8
w-NN	8.03	—	7.90	—
NNa	3.19	37.5	3.20/3.14	37.5
NNb	1.62	26.6	1.60	26.6
NNc	2.30	57.0	2.29	57.0
NNd	2.17	44.9	2.17	44.9
AG-NH	7.74	—	7.69	—
AG1	5.32	102.1	5.17	102.3
M1	5.25	96.8	5.05	98.2

^1H NMR Spectra of the Other Compounds

The ^1H NMR spectra of the other derivatives of A-40,926 were compared with that of RA-A-1. Significant assignments for some selected compounds are given in Table 5.

The absence of mannose in the demannosyl (DM) derivatives was confirmed by the upfield shifts of protons

Table 5. Significant ^1H NMR assignments for A and selected derivatives.

Compound	Proton (chemical shift, δ ppm)						
	x2	x3	x4	x5	x6	x7	z6
A	4.84	6.09	5.49	4.41	4.08	4.50	5.06
A-A-1[a]	4.87	6.09	5.62	4.42	4.15	4.35	5.25
MA-A-1[a]	4.80	6.09	5.58	4.43	4.18	4.33	5.35
MA-A-3	4.85	6.10	5.62	4.44	4.13	4.41	5.15
RA	4.90	6.09	5.62	4.41	4.12	4.49	5.09
RA-A-1	4.91	6.08	5.65	4.42	4.13	4.41	5.19
RA-A-2[a]	4.95	6.08	5.60	4.42	4.16	4.38	5.25
RA-A-4	4.88	6.07	5.60	4.30	4.07	4.38	5.08
DMA	4.95	6.06	5.60	4.32	4.11	4.39	5.07
DMA-A-1	4.95	6.07	5.62	4.34	4.16	4.29	5.26
DMMA-A-1	4.98	6.07	5.62	4.34	4.16	4.32	5.19
DMRA	4.95	6.06	5.60	4.31	4.10	4.40	5.07
DMRA-A-1[a]	4.95	6.06	5.63	4.33	4.18	4.30	5.22
PyMA-A-1	4.96	6.14	5.65	4.34	4.14	4.34	5.20
PyRA-A-1	4.97	6.14	5.65	4.37	4.18	4.37	5.20

[a] Salified with an external anion (by adding TFA).

Table 6. Significant ionization constants for some representative compounds (determined in MCS*-H_2O 4:1 solution).

Compound	pK_{MCS}[a]	Attribution
A	4.4	COOH (acyl sugar)
	5.5	COOH (heptapeptide chain)
	7.2	$NHCH_3$
	9.2	OH (phenolic ring 3)
RA-A-1	7.2	$NHCH_3$
	8.2	$N(CH_3)_2$
	9.2	OH (phenolic ring 3)
PyRA-A-1	—[b]	$NHCH_3$
	8.2	$N(CH_3)_2$
	9.2	P^+
PyA-A-1	4.9	COOH
	6.7	$NHCH_3$
	8.2	$N(CH_3)_2$
	9.2	P^+

[a] Methyl Cellosolve.
[b] Not detectable due to lack of a suitable resolution of the titration curve.

7d (from 6.71 to 6.37 ppm) and 7f (from 6.42 to 6.23 ppm).[17] The presence of the methyl ester moiety in MA-derivatives is shown by the OCH_3 signal at δ 3.63 ppm.

A common feature of the amide derivatives, compared with corresponding unmodified compounds, is the downfield shift of the z6 signal by $\sim$0.2 ppm which is in accordance with formation of the amide.[18] The chemical shifts of the basic moieties of the amide chains change according to the ionization status of the molecule. In particular, in the A-1 amides the $N(CH_3)_2$ signal at δ 2.17 ppm (free base) is shifted to 2.50$\sim$2.71 ppm when it is salified with an external anion. The protons of the amide chain in the salified A-2-type derivatives give signals at δ 2.95 and 1.85 ppm. As the free bases, the protons of the amide chain of the A-3 and A-4 compounds resonate at δ 2.81 and 1.73 ppm, and at δ 2.11 ppm (*N*-methylpiperazine), respectively.

NMR Spectroscopy of Py-Derivatives

The structures of the *tris*-pyrrolidinophosphonium (Py) derivatives were determined by ^1H and ^{31}P NMR spectroscopies.

The presence of the *tris*-pyrrolidino moiety was established by TOCSY experiments, while the phosphoryl phosphorus was detected by ^{31}P NMR using triethylphosphate (TEP) as reference (δ_{TEP} 0 ppm). The ^{31}P signal was found at δ 25.4 ppm. In particular, in the ^1H NMR spectrum of PyRA-A-1 compared with that of RA-A-1, the downfield shift of the x3 ($\Delta\delta$ 0.06 ppm) and 3d ($\Delta\delta$ 0.07 ppm) protons indicated[†9] that the phosphonium residue is linked with the phenolic oxygen of ring 3. Additionally, the $\Delta\delta$ value for x3 is more pronounced (0.17 ppm) when PyRA-A-1 is salified with an external anion.

Acid-base Ionization

The ionization status of some of the amide derivatives of A-40,926 was determined by acid-base titration. The titration curves were interpreted on the basis of the knowledge of the relationships between ionization properties and functional groups present in the glycopeptides of the teicoplanin family.[3] For compounds RA-A-1, PyRA-A-1 and PyA-A-1 the pK_{MCS} values of the most significant ionizable functions and their assignments are given in Table 6, in comparison with those of A.

Antibacterial Activity

Minimum inhibitory concentrations (MICs) were determined by broth microdilution[19] using inocula of 5×10^5 CFU/ml and incubation at 37°C. Staphylococci and enterococci were grown in Iso-Sensitest broth (Oxoid), streptococci in Todd-Hewitt broth (Difco) and *Neisseria gonorrhoeae* in GC Base broth (Difco)+1% Iso VitaleX (BBL). *N. gonorrhoeae* was incubated for 48 hours in 5% CO_2; all other organisms were incubated for 20$\sim$24 hours in air.

With the exception of its activity against *N. gonor-*

[†9] In analogy with the variation of the chemical shift of protons 7d and 7f in the presence or absence of the mannose on ring 7.[17]

rhoeae, A-40,926 (A) generally has activity similar to that of teicoplanin, although it tends to be slightly less active than teicoplanin against some staphylococcal isolates[14] and shows slight activity (Table 9 and unpublished data) against some VanA enterococci, which are generally more resistant to teicoplanin and always more resistant to vancomycin. The A-amides and some of the MA-amides were significantly more active than A (Tables 7 and 8) against staphylococcal isolates chosen on the basis of their reduced susceptibility to A. These amides are also significantly more active than teicoplanin against these isolates (Data not shown); in this respect they behave in a similar manner to certain amides of teicoplanin.[18,20] Most of the RA-amides also had significantly better activity against the selected staphylococci and, in some cases, also had somewhat better activity against streptococci (Table 9). However, the most novel feature of the amides, which was particularly evident for some of the MA- and RA-amides, was the moderate activity that they displayed (MIC as low as

Table 7. *In vitro* antibacterial activity of A-40,926 (A) and A-amides.

Organism	MIC (μg/ml)			
	A	A-A-1	A-A-2	A-A-3
Staphylococcus aureus Tour	0.13	0.13	0.06	0.06
S. aureus clinical isolate	8	0.5	0.13	0.13
S. epidermidis ATCC 12228	8	0.25	0.06	0.13
S. epidermidis clinical isolate	16	0.13	0.03	0.06
S. haemolyticus clinical isolate	32	0.13	0.13	0.06
Streptococcus pyogenes C203	0.06	0.03	0.5	0.03
S. pneumoniae UC41	0.06	0.06	0.13	0.03
Enterococcus faecalis ATCC 7080	0.13	0.13	0.06	0.13
E. faecalis clinical isolate	64	16	32	16
Neisseria gonorrhoeae ISM68/126	1	1	8	8

Table 8. *In vitro* antibacterial activity of MA-derivatives.

Organism	MIC (μg/ml)					
	MA	MA-A-1	MA-A-2	MA-A-3	MA-A-4	MA-A-5
Staphylococcus aureus Tour	0.25	0.06	0.06	0.13	0.06	0.06
S. aureus clinical isolate	8	4	0.13	4	0.25	0.13
S. epidermidis ATCC 12228	4	1	0.13	1	0.13	0.13
S. epidermidis clinical isolate	4	0.13	0.13	2	0.06	0.06
S. haemolyticus clinical isolate	16	1	0.06	2	0.13	0.06
Streptococcus pyogenes C203	0.06	0.06	0.03	0.06	0.13	0.03
S. pneumoniae UC41	0.06	0.016	0.06	0.06	0.06	0.03
Enterococcus faecalis ATCC 7080	0.25	0.06	0.13	0.13	0.13	0.13
E. faecalis clinical isolate	64	8	8	8	16	16
Neisseria gonorrhoeae ISM68/126	2	16	32	16	32	8

Table 9. *In vitro* antibacterial activity of RA-derivatives.

Organism	MIC (μg/ml)					
	RA	RA-A-1[a]	RA-A-2	RA-A-3	RA-A-4	RA-A-5
Staphylococcus aureus Tour	0.06	0.06	0.13	0.06	0.06	0.06
S. aureus clinical isolate	4	0.13	0.13	0.13	2	0.13
S. epidermidis ATCC 12228	2	0.13	0.13	0.06	0.25	0.13
S. epidermidis clinical isolate	2	0.06	0.06	0.06	0.13	0.06
S. haemolyticus clinical isolate	16	0.13	0.06	0.13	0.5	0.13
Streptococcus pyogenes C203	0.03	0.06	0.06	0.03	0.03	0.13
S. pneumoniae UC41	0.016	0.016	0.03	0.016	0.03	0.13
Enterococcus faecalis ATCC 7080	0.13	0.06	0.06	0.13	0.06	0.13
E. faecalis clinical isolate	16	8	8	8	8	32
Neisseria gonorrhoeae ISM68/126	8	16	64	32	16	64

[a] Median of 7 determinations.

8 μg/ml) against a VanA strain of *Enterococcus faecalis* (Tables 7 and 9).

The demannosyl pseudoaglycone (DMA) of A-40,926 was previously reported to have enhanced activity against certain staphylococcal isolates, as compared with A.[14] This was confirmed (Tables 7 and 10), and was also observed for two DMA derivatives (Table 10). However, the amides of these compounds were even more active against some isolates (Table 10) and had activity comparable to those of the amide derivatives discussed above (Tables 7~9). Among the DM-derivatives, DMMA-A-1 and DMRA-A-1 were particularly active against the VanA enterococcal strain (MICs 4 μg/ml). The amide derivative (DMGA-A-1) of A-40,926 aglycone also had excellent activity against staphylococci, although the aglycone itself was previously reported to be less active than DMA against some isolates of this type.[14]

Among all of the A-40,926 derivatives, RA-amides MDL 63,246 (RA-A-1) and MDL 63,042 (RA-A-2) were selected for additional studies of their anti-enterococcal activity, in part because they were found to be particularly active against experimental septicemia in mice (Unpublished data). As shown in Table 11, the MIC distribution of these compounds against VanA enterococci was rather broad; however, MICs of RA-A-2 were as low as 0.5 μg/ml against some isolates. Both compounds were at least as active as teicoplanin against teicoplanin-susceptible enterococci (vancomycin-susceptible, VanB, and VanC strains).

The antibacterial activity of the pyrrolidinyl by-products PyA-A-1, PyMA-A-1 and PyRA-A-1 (Table 12) was similar to that of the corresponding pyrrolidino-free amides against streptococci; however, the pyrrolidinyl compounds were significantly less active against staphylococci. Although they were 4 to 16 fold less active than the parent amides A-A-1, MA-A-1 and RA-A-1 against a glycopeptide-susceptible strain (L149) of *E. faecalis*, PyA-A-1, PyMA-A-1 and PyRA-A-1 had comparable or better activity against the glycopeptide-resistant strain L562.

Most of the derivatives were less active than A-40,926 against *N. gonorrhoeae*.

Structure-Activity Relationships

In teicoplanin-like antibiotics, activity against highly glycopeptide-resistant (VanA) enterococci correlated with the loss of the 34-acetylglucosamine, as the amide derivatives of A-40,926 and of 34-deacetylglucosaminyl teicoplanin (DAGT)[4] were both active against these isolates, while teicoplanin amides were inactive.

The presence or absence of mannose had little or no

Table 10. *In vitro* (MIC) activity of demannosyl compounds (DM-derivatives) and aglycone DMGA-A-1.

Organism	MIC (μg/ml)						
	DMA	DMMA	DMRA	DMA-A-1	DMMA-A-1	DMRA-A-1	DMGA-A-1
Staphylococcus aureus Tour	0.06	0.06	0.06	0.06	0.06	0.06	0.13
S. aureus clinical isolate	0.25	1	0.25	0.13	0.06	0.13	0.13
S. epidermidis ATCC 12228	2	1	1	0.06	0.13	0.06	0.13
S. epidermidis clinical isolate	0.13	0.13	0.25	0.03	0.03	0.06	0.13
S. haemolyticus clinical isolate	0.5	2	1	0.13	0.5	0.25	0.25
Streptococcus pyogenes C203	0.03	0.06	0.06	0.06	0.03	0.03	0.5
S. pneumoniae UC41	0.06	0.016	0.03	0.03	0.03	0.06	0.25
Enterococcus faecalis ATCC 7080	0.25	0.13	0.06	0.06	0.13	0.13	0.13
E. faecalis clinical isolate	32	8	16	32	4	4	>128
Neisseria gonorrhoeae ISM68/126	4	16	8	8	64	128	32

Table 11. *In vitro* activity against enterococci.

Class (no. isolates)	MIC range (MIC_{50}) (μg/ml)			
	RA-A-1	RA-A-2	Teicoplanin	Vancomycin
Susceptible (11)	0.016~0.13 (0.06)	0.03~0.25 (0.06)	≤0.13~0.5 (0.13)	1~8 (2)
VanA (19)	4~64 (16)	0.5~32 (8)	64~>128 (>128)	>128
VanB (7)	0.13	0.06~0.5 (0.13)	0.13~1 (0.25)	32~>128 (>128)
VanC (5)	0.06~0.13	0.03~0.5	0.25~0.5	4~8

Table 12. *In vitro* antibacterial activity of Py-amides.

Organism	MIC (μg/ml)		
	PyA-A-1	PyMA-A-1	PyRA-A-1
L165 *Staphylococcus aureus* Tour	0.25	1	1
L561 *S. aureus* clinical isolate	4	16	16
L147 *S. epidermidis* ATCC 12228	2	8	4
L533 *S. epidermidis* clinical isolate	1	4	4
L602 *S. haemolyticus* clinical isolate	2	8	8
L49 *Streptococcus pyogenes* C203	0.06	0.13	0.06
L44 *S. pneumoniae* UC41	0.06	0.06	0.06
L149 *Enterococcus faecalis* ATCC 7080	0.5	0.5	1
L562 *E. faecalis* clinical isolate	4	8	8
L997 *Neisseria gonorrhoeae* ISM68/126	128	>128	>128

influence on activity against VanA enterococci, while the presence of the *N*-acylglucosamine (RA or DAGT amides), or *N*-acylglucuronic acid (A amides) or its methyl ester (MA amides) was essential for the activity against these bacterial strains.

The removal of mannose from RA-amide MDL 63,246 (RA-A-1) to give DMRA-A-1 had no effect on activity against a VanA strain of *Enterococcus faecalis* (L562) but the aglycone DMGA-A-1 of MDL 63,246 had no activity against this strain. As these two compounds had similar activity against a vancomycin-susceptible strain (L149) of *E. faecalis*, the *N*-acylglucosamine appears to be specifically required for activity against VanA enterococci, rather than for general anti-enterococcal activity.

Conclusion

The basic amides of A-40,926 (A), its 6^B-methyl ester (MA) and 6^B-decarboxy-6^B-hydroxymethyl derivative (RA) had moderate *in vitro* activity against isolates of *Enterococcus faecalis* and *E. faecium* which are highly resistant to vancomycin and teicoplanin.

Most of these compounds, in particular RA-amides, had excellent activity against all Gram-positive bacteria tested, including VanB and VanC enterococci and, unexpectedly,[†10] against staphylococci with reduced susceptibility to teicoplanin and A-40,926.

Among the RA-amides, MDL 63,246 and MDL 63,042 were selected for further investigation of their antibacterial properties. They were more active than other glycopeptide antibiotics against staphylococci and several other groups of Gram-positive bacteria.

Experimental

NMR spectra were obtained with a Bruker AMX 600 instrument. The spectra were recorded at 303 K in DMSO-d_6 solution. The data were processed on an Aspect station with the UXNMR software from Bruker. All homonuclear experiments (DQF-COSY, TOCSY and ROESY) were acquired with a spectral width of 11 ppm. In all of the experiments, spectra were recorded with 512 increments in t_1 and 4096 complex data points in t_2. For the ROESY 32 transients were averaged for each t_1 value, for COSY and TOCSY 16 transients. Mixing times of 70 or 150 ms were used for TOCSY and ROESY/NOESY spectra respectively. For HMQC spectra 512 increments with 2048 complex data points in t_2 were collected using a sweep width of 11 ppm in the proton and 165 ppm in the carbon dimension. A BIRD pulse[21] was applied to suppress magnetization of protons connected to ^{12}C (recovery delay of 200 ms). The HMBC spectra were acquired with a sweep width of 11 ppm in the proton and 165 ppm in the carbon dimension. A total of 320 transients were averaged for each of 512 increments in t_1, and 2048 complex points in t_2 were recorded. A delay of 3.3 ms was used to supress 1-J couplings and 70 ms were taken for the development of long range correlations. After Fourier transformation the strong t_1 noise was reduced by a mean row subtraction using the AURELIA program (Bruker).

Phosphorus Determination in P-containing Compounds and in Samples of RA-A-1

The ^{31}P NMR experiments for the phosphorus determination in the Py-derivatives were run using the Powgate (Bruker) microprogram (CPD decoupling, 11.5 μs for 90° pulse) with exponential multiplication (LB=3.3 Hz) prior to Fourier transformation. The ^{31}P NMR spectra were recorded in DMSO-d_6 solution at 30°C at 202.46 MHz with a Bruker AM 500 NMR spectrometer (Aspect 3000 console).

The use of PyBOP as condensing agent in the synthesis of the amide derivatives produced phosphorus-contain-

[†10] The excellent anti-staphylococcal activity of most of the amide derivatives of A-40,926 was unexpected because analog amides of de-acetylglucosaminyl teicoplanin were generally less active than the corresponding amides of teicoplanin against *S. aureus* and coagulasenegative staphylococci.

ing by-products such as *tris*-pyrrolidinophosphonium (Py) compounds, *tris*-(*N*,*N*-tetramethylene)-phosphoric acid triamide (TPPA), and PF_6^-. All three side-reaction products, which are potential impurities in final amidation derivatives, can be separately detected by ^{31}P NMR. Their P-chemical shifts are as follows: δ 14.7 ppm for TPPA, δ −143.1 ppm for PF_6^-, and δ 25.4 ppm, as previously mentioned, in the case of PyRA-A-1. The quantitative NMR analysis is based on the measurement of the integrals of the ^{31}P-resonances corresponding to the above impurities, using weighted amounts of $(EtO)_3PO$ (TEP) as internal references. The standard solution was prepared by dissolving 1 mg of TEP in 10 ml of DMSO-d_6. The sample solution was prepared by dissolving 20 mg of each compound in 1 ml of the standard solution. The percentages (by weight) of the P-containing impurities were calculated as follows:

$$\% \text{ of impurity i in the sample} = \frac{w_s \times 17.00 \times H_i \times 10}{H_s \times P_i \times w_E}$$

where: w_s = weight of TEP standard in mg
w_E = weight of the sample in mg
H_s = integral (instrumental arbitrary unit) of TEP resonance
H_i = integral of the resonance of impurity i
17.00 = % of P in TEP
$\%P_i$ = % of P in the impurity i (*i.e.*, 12.03% in TPPA, 21.37% in PF_6^-, 1.516% in PyRA-A-1)

Each amide derivative contained no more than 0.2% (by weight) of the corresponding Py-amide as the main impurity, while TPPA or PF_6^- were generally absent.

FAB-MS positive ion spectra were obtained on a Kratos MS-50 instrument fitted with a standard FAB source and a high-field magnet. The sample (~10 nmol) was dispersed in a few microliters of α-thioglycerol-diglycerol 1:1 matrix and bombarded with a 6~9 keV beam of Xe atoms.

The products were purified by reversed-phase column chromatography on silanized silica gel (0.063~0.2 mm; Merck). Reactions, column eluates, and final products were checked by HPLC performed on a Hibar column (125×4 mm; Merck) prepacked with LiChrospher RP-8 (5 μm), using a Varian Model 5500 LC pump equipped with a Rheodyne Model 7125 20-μl loop injector and a Varian Model 2050 UV variable detector. Chromatograms were recorded at 254 nm. Elutions were carried out at a flow rate of 1.5 ml/minute according to a linear gradient from 20% to 60% of CH_3CN in 0.2% aqueous HCO_2NH_4 in 30 minutes.

All derivatives were analyzed for N and Cl, on samples previously dried at 140°C under N_2 atmosphere. Weight loss was determined by thermogravimetry (TG), at 140°C. Inorganic residue was determined after heating the samples at 900°C in O_2 atmosphere. The analytical results obtained for N and Cl were within ±0.4% of the theoretical values. Solvent content (in general H_2O, with traces of BuOH) and inorganic residue were always less than 10% and 0.3%, respectively.

Most of the intermediate di- and polyamines are commercially available products which were purchased from Fluka-Chemie AG or Aldrich-Chemie Gmbh & Co. KG, with the exception of the branched tetramine $N[(CH_2)_3NH_2]_3$, used in the synthesis of amides A-, MA- and RA-A-3, which was prepared according to a procedure previously described.[20]

A-40,926 6^B-Methyl Ester (MA)

A suspension of 1.75 g (~1 mmol) of A in 250 ml of MeOH was adjusted at pH 2 with concentrated H_2SO_4, obtaining a solution which was stirred at room temperature for 26 hours. The reaction mixture was brought at pH ~6 with triethylamine and then Et_2O (250 ml) was added. The precipitated solid was collected, washed with H_2O (50 ml) and then dried at room temperature *in vacuo* overnight, yielding 1.75 g of title compound.

6^B-Decarboxy-6^B-hydroxymethyl (RA) A-40,926 (Method A)

To a stirred solution of 1.75 g (~1 mol) of MA and 1 g of $NaHCO_3$ in 50 ml of a water-dioxane (1:1) mixture, a solution of 0.25 g (~1.15 mmol) of di-*tert*-butyl dicarbonate (BOC_2O) in 5 ml of dioxane was added dropwise at 5°C within 15 minutes. After 1 hour at room temperature, the reaction mixture was adjusted at pH 4 with 1 N HCl and 150 ml of H_2O was added. The resulting mixture was extracted with BuOH (2×100 ml), the organic layer was washed with 100 ml of H_2O and then it was concentrated to a small volume (~25 ml) at 40°C under reduced pressure. On adding Et_2O (100 ml) the precipitated solid (1.6 g, BOC-MA) was collected and suspended in 150 ml of a H_2O-BuOH (2:1) mixture. Under vigorous stirring, 1.6 g of $NaBH_4$ was added portionwise at room temperature over 30 minutes. The resulting solution was stirred at room temperature for 1 hour, then it was cooled at 5°C and 27 ml of glacial AcOH was added followed by 100 ml of H_2O. Extraction with BuOH (200 ml) and evaporation of the organic solvent, at 40°C under reduced pressure, yielded a solid residue (1.5 g, BOC-RA) which was dissolved in 15 ml of TFA previously cooled at 0°C. After stirring at 0~5°C for 25 minutes, the resulting solution was poured into 30 ml of a Et_2O-MeOH (4:1) mixture at 0°C. The precipitated solid was collected, washed several times with Et_2O, and then it was dissolved in 100 ml of H_2O. The resulting solution was adjusted at pH 5.5 with 0.1 N NaOH and loaded on a column of 100 g of silanized silica-gel in a H_2O-MeCN (9:1) solution. Elution was performed by a linear step gradient from 10 to 50% of MeCN in H_2O in 15 hours at a flow rate of 200 ml/hour while collecting 15 ml fractions. Those containing pure title compound were pooled and solvents were evaporated at 35°C, under reduced pressure, in the presence of enough BuOH to prevent foaming. The solid residue was collected, washed with Et_2O (50 ml) and then

dried *in vacuo* at room temperature overnight, yielding 1.05 g of the title compound.

Preparation of DMA, or DMMA, by Demannosylation of A, or MA

A solution of 10 mmol of A, or MA, and of 15 ml of 37% HCl in 100 ml of dimethylsulfoxide (DMSO) was stirred at 55~60°C for 15 hours. The reaction mixture was cooled at room temperature and then it was poured into 350 ml of H_2O and extracted with BuOH (350 ml). The organic layer was washed with H_2O (2 × 100 ml) and concentrated at 50°C under reduced pressure to a small (~30 ml) volume. On adding EtOAc (170 ml) the precipitated solid was collected and dried at room temperature *in vacuo* overnight, yielding the title compounds.

Preparation of DMRA (Method B)

To a stirred solution of 5 g (~3 mmol) of DMMA in 150 ml of a H_2O-BuOH (2:1) mixture, 1.5 g of $NaBH_4$ was added portionwise at room temperature in 30 minutes. After stirring at room temperature for additional 4 hours, the reaction mixture was cooled at 5°C and adjusted at pH 3.5 with glacial AcOH. After adding 300 ml of a BuOH-H_2O (3:1) mixture, the organic layer was separated, washed with 100 ml of H_2O and concentrated at 35°C under reduced pressure to a small (~25 ml) volume. On adding 150 ml of EtOAc the precipitated solid was collected and purified by reversed-phase column chromatography as described above for RA, yielding 0.75 g of the title compound.

Preparation of MA- and RA-amides (General Procedure)

To a stirred solution of 1 mmol of MA, or RA, in 30 ml of DMSO, 1.15 mmol of the appropriate amine and 600 mg (~1.2 mmol) of PyBOP were added at room temperature. After stirring at 20~25°C for 3 hours, 150 ml of Et_2O was added and the precipitated solid was collected. Purification by reversed-phase column chromatography was performed with a linear gradient from 10 to 75% of MeCN in 0.1 N AcOH. Fractions containing (HPLC) pure title compounds were pooled and worked-up as usual, yielding pure MA- or RA-amides.

Preparation of A-amides (General Procedure)

To a stirred suspension of 2.5 mmol of the corresponding MA-amide in 60 ml of THF, 10 ml of H_2O and 20 ml of 1 N NaOH were added at room temperature. After 30 minutes, the resulting solution was adjusted at pH 7 with 1 N HCl, 150 ml of BuOH was added and the resulting mixture was concentrated at 40°C underreduced pressure to a small (~20 ml) volume. On adding Et_2O (200 ml) the precipitated solid was collected and purified by reversed-phase column chromatography under the same conditions described above for the MA- and RA-amides, yielding pure title compounds.

Preparation of DMMA-A-1

To a stirred solution of 5 g (~3 mmol) of DMMA in 50 ml of DMSO, 0.5 ml (~4 mmol) of 3,3-dimethylamino-1-propylamine was added at room temperature followed by 1 g of PyBOP. After 30 minutes, EtOAc (250 ml) was added and the precipitated solid was collected (5.1 g) and purified by reversed-phase column chromatography as described above for MA- and RA-amides, yielding 2.5 g of the title compound.

Preparation of DMA-A-1

To a stirred solution of 1.7 g (~1 mmol) of DMMA-A-1 in 35 ml of a THF-H_2O (6:1) mixture, 5 ml of 1 N NaOH was added at room temperature. After 10 minutes, the reaction mixture was adjusted at pH 3 with 1 N HCl and extracted with BuOH (100 ml). The organic layer was separated, washed with H_2O (2 × 25 ml) and concentrated at 35°C under reduced pressure to a small (~5 ml) volume. On adding EtOAc (25 ml) the precipitated solid was collected, washed with Et_2O and dried at room temperature *in vacuo* overnight, yielding 0.75 g of the title compound.

Preparation of DMRA-A-1 (Method B)

To a stirred solution of 1.7 g (~1 mmol) of DMMA-A-1 in 70 ml of a H_2O-BuOH (2:1) mixture, 0.5 g of $NaBH_4$ was added at room temperature in 20 minutes. Stirring was continued for 2 hours, then the reaction mixture was cooled at 5°C and adjusted at pH 5 with glacial AcOH. The resulting solution was extracted with BuOH (150 ml). The organic phase was separated, washed with H_2O (50 ml) and then it was concentrated at 40°C under reduced pressure to a small (~10 ml) volume. On adding Et_2O (100 ml) the precipitated solid was collected (1.1 g) and purified by reversed-phase chromatography under usual conditions, yielding 0.35 g of title compound.

Preparation of DMGA-A-1

A solution of 1.7 g (~1 mmol) of DMRA-A-1 in 15 ml of dry TFA was stirred at room temperature for 1 hour. The solvent was evaporated at room temperature under reduced pressure, the oily residue was dissolved in 5 ml of MeOH and then 20 ml of EtOAc was added. The precipitated solid was collected and purified by reversed-phase chromatography, yielding 0.47 g of title compound.

Preparation of PyMA-A-1 and PyRA-A-1

To a stirred solution of 1 mmol of MA, or RA, in 30 ml of DMSO, 3 mmol of 3,3-dimethylamino-1-propylamine and 3 mmol of PyBOP were added at room temperature. After 1 hour, 150 ml of Et_2O was added and the precipitated solid was collected. Purification was by reversed-phase column chromatography, eluting with a linear gradient from 10 to 90% of MeCN in 0.1 N AcOH. Fractions containing pure (HPLC) title com-

pounds were pooled and worked up as usual, yielding pure PyMA-A-1, or PyRA-A-1, as the di-acetates.

Preparation of PyA-A-1

This compound was obtained, as the mono-acetate, from PyMA-A-1 under the same conditions described above for the preparation of A-amides from MA-amides.

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