Synthesis and Antibacterial Activity of Derivatives of the Glycopeptide Antibiotic A-40926 and Its Aglycone

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Starting from the antibiotic A-40926 and the aglycone of A-40926 a series of compounds were prepared by modifying the free functionalities. Their antimicrobial activity was determined, particularly against *Neisseria gonorrhoeae*, against which A-40926, unlike other natural glycopeptides, is active. Improved *in vivo* activity was displayed by the monomethyl ester of A-40926 esterified at the carboxyl group of the *N*-acylamino-glucuronyl moiety.

A-40926 is a glycopeptide antibiotic produced by an actinomycete of the genus *Actinomadura*[1]. It was discovered during a screening campaign for glycopeptide antibiotics using specific binding to D-alanyl-D-alanine affinity resins[1,2]. This suggests that its mode of action is similar to that of glycopeptide antibiotics of the vancomycin group which inhibit cell wall biosynthesis by binding to the terminal D-alanyl-D-alanine residues of the pentapeptide side-chain of nascent peptidoglycan[3]. Independently, another group isolated A-40926 from *Actinomadura parvosata*[4]. The *in vitro* activity of A-40926 against aerobic and anaerobic Gram-positive bacteria is similar to that of other glycopeptide antibiotics such as vancomycin[5], teicoplanin[6], aridicins[7], kibdelins[8], chloropolysporins[9]. However, unlike these antibiotics it is active against the Gram-negative bacterium *Neisseria gonorrhoeae*[1]. A-40926 is composed of two main factors, A and B, which differ in the fatty acid chains attached to a 2-amino-glucuronic acid moiety. In factor A (9%) the chain is due to *n*-undecanoic acid while in B (82%) is 10-methylundecanoic acid. In this paper we will refer, for simplicity, to factor B only, due to its prevailing abundance. The peptide core is identical in both factors, which also contain D-mannose (Fig. 1). The terminal amino group is monomethylated[10].

Due to the interesting activity of A-40926 against *Neisseria gonorrhoeae*, a chemical modification program was planned with the aim of improving its activity against this species and other Gram-negative bacteria and obtaining information about structure-activity relationships. For this purpose modifications were made in order to change such physicochemical properties such as pK_a and lipophilicity.

Chemistry

The structure of A-40926 (Fig. 1) suggested three functionalities as targets for chemical derivatization: the monomethylated amino group in position 15, the free carboxyl group in position 33, and the free carboxyl group of the *N*-acylaminoglucuronyl moiety.

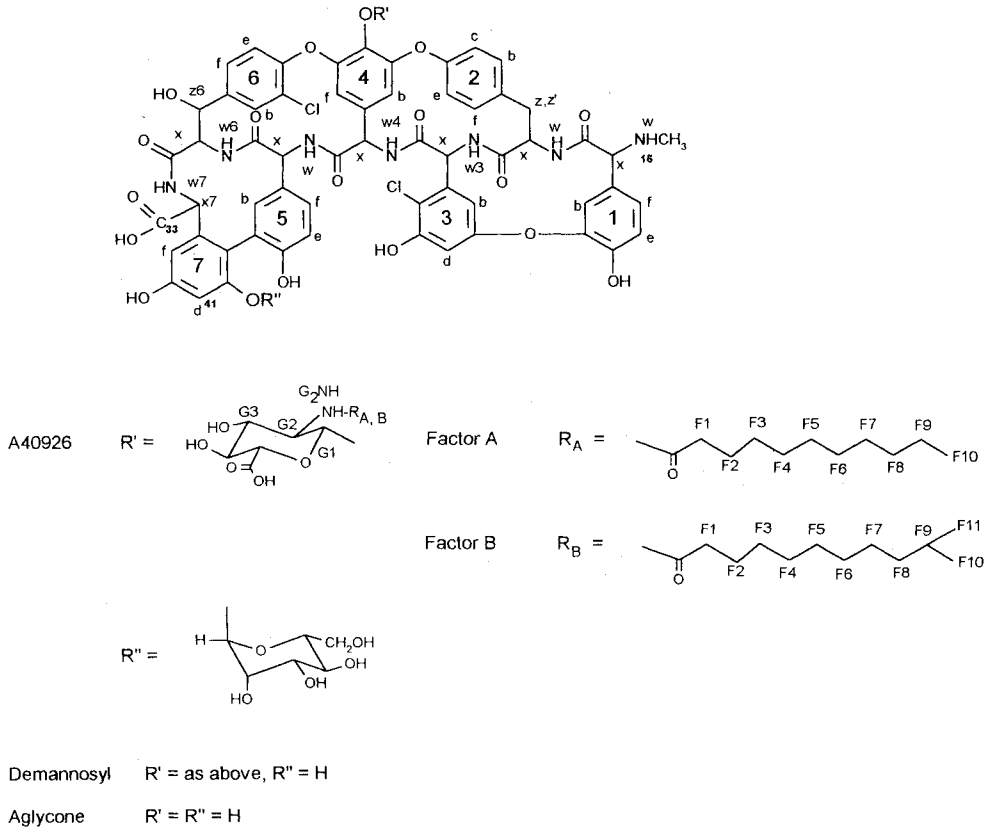
As even simple reactions are difficult to carry out with glycopeptide antibiotics, and since the carboxyl group of the *N*-acyl-aminoglucuronic acid moiety could complicate selective derivatization reactions of the carboxyl group at position 33 of the heptapeptide core, we first developed a suitable method for removing all of the carbohydrate moieties from the heptapeptide core.

By dissolving A-40926 in DMSO containing 5% aqueous HCl and heating the resulting clear solution for 9.5 hours at +80°C A-40926 aglycone (Fig. 1, R′= R″=H) was obtained, after column separation, with a yield of 86% in the form of large crystals (Scheme 1)[11]. Other hydrolysis conditions, *e.g.*, $(CH_3)_3SiCl/NaI$ led only to slow decomposition of the starting material. Starting from this aglycone a series of derivatives were prepared (Table 1).

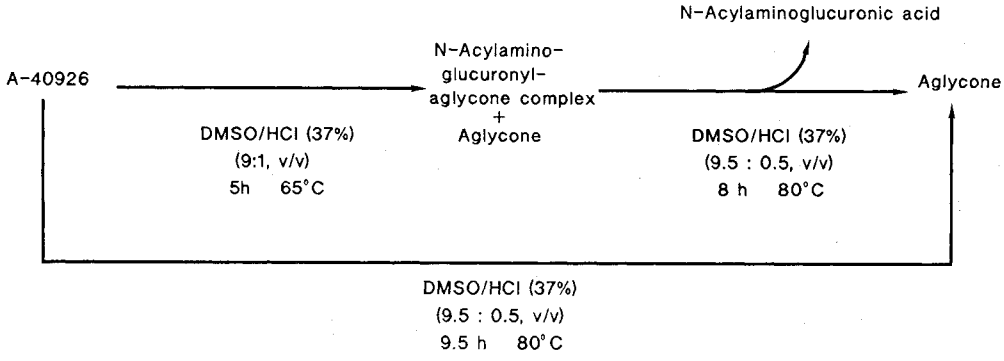
Derivatization of the Amino Group

Some synthetic work was carried out to explore the reactivity of the monomethylated amino group by

Fig. 1. Structures of A-40926 and of its aglycones.



Scheme 1.



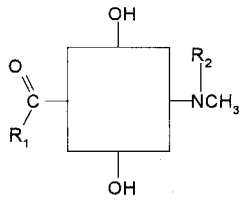
derivatization reactions which changed charge and basicity.

N-acetylation was achieved with acetic anhydride at low temperature yielding compound I (Table 1).

Maleic acid anhydride reacted with opening of the anhydride ring and amide formation to a compound II, which contains a α,β-unsaturated carboxylic acid.

Reaction with chlorosulfonic acid should lead to the introduction of a sulfate group. The attachment of this group was potentially interesting because, among all reported glycopeptides, there is one, A-47934[13], an aglycone with a sulfonated phenolic hydroxyl group, which is more active against *Neisseria gonorrhoeae* than the aglycone of A-40926 (unpublished data). Thus, sulfonation of a phenolic hydroxyl group might enhance anti-*Neisseria* activity. With the unprotected aglycone clean *N*-sulfonation was obtained (compound III). Treatment of the *N*-protected aglycone or of A-40926 itself produced inseparable mixtures. Attempts to introduce a sulfate group by biotransformation of the

Table 1. Derivatives A-40926 aglycone.



Compound	R_1	R_2	Yield (%)	Formula	MH (calc.)	MH (FAB, found)
I	OH	$COCH_3$	99	$C_{61}H_{49}Cl_2N_7O_{19}$	1254.3	1254.3
II	OH	$COCH{=}CHCO_2H$	88	$C_{63}H_{49}Cl_2N_7O_{21}$	1310.2	1310.2
III	OH	SO_3H	99	$C_{59}H_{47}SCl_2N_7O_{21}$	1292.2	1314.2[b]
IV	OH	CH_3	26	$C_{60}H_{49}Cl_2N_7O_{18}$	1226.3	1226.3
V	OH	Boc(t)	14[a]	$C_{64}H_{55}Cl_2N_7O_{20}$	1312.3	1312.3
VI	OH	Bzl	50[a]	$C_{66}H_{53}Cl_2N_7O_{18}$	1302.3	1302.4
VII	OCH_3	H	73	$C_{60}H_{49}Cl_2N_7O_{18}$	1226.3	1226.4
VIII	OC_2H_5	H	99	$C_{61}H_{51}Cl_2N_7O_{18}$	1240.3	1240.1
IX	OC_3H_7	H	99	$C_{62}H_{53}Cl_2N_7O_{18}$	1254.3	1254.4
X	$O(CH_2)_2OH$	H	32[a]	$C_{61}H_{51}Cl_2N_7O_{19}$	1256.3	1257 (±2)
XI	$O(CH_2)_4OH$	H	59[a]	$C_{63}H_{55}Cl_2N_7O_{19}$	1284.3	1286 (±2)
XII	OC_3H_6Br	Bzl	18[a]	$C_{69}H_{58}BrCl_2N_7O_{18}$	1422.3	1422.3
XIII	OC_3H_6Br	H	73[a]	$C_{62}H_{52}BrCl_2N_7O_{18}$	1332.2	1332.2
XIV	OCH_2CN	Bzl	97	$C_{68}H_{54}Cl_2N_8O_{18}$	1341.3	1341.4
XV	NH_2	Bzl	38	$C_{66}H_{54}Cl_2N_8O_{17}$	1301.3	1301.3
XVI	NH_2	H	58	$C_{59}H_{48}Cl_2N_8O_{17}$	1211.3	1213 (±2)
XVII	$NHCH_3$	Bzl	13[a]	$C_{67}H_{56}Cl_2N_8O_{17}$	1315.3	1314.9
XVIII	$NHCH_3$	H	34	$C_{60}H_{50}Cl_2N_8O_{17}$	1225.3	1225.4
XIX*	OH	H	11[a]	$C_{59}H_{46}BrCl_2N_7O_{18}$	1290.1	1290.2
XX**	OH	H	34[a]	$C_{59}H_{45}Br_2Cl_2N_7O_{18}$	1368.1	1368.1

[a] After preparation HPLC.
[b] $M^+ + Na$.
* Contains 1 bromine atom at position 41.
** Y contains 1 bromine atom at position 41 and a second bromine atom.

N-protected or unprotected aglycone in the presence of the strain which produces A-47934 were unsuccessful.

Other reactions carried out were *N*-formylation with HCO_2H/Ac_2O and *N*-methylation with HCHO/Na-BH_3CN. The *N*-formyl compound was detectable by HPLC but hydrolyzed completely during column purification.

Protection of the amino group with benzyl chloroformate under various experimental conditions gave rise to mixtures of compounds. Protection with *t*-butyl-2,4,5-trichloro-phenylcarbonate using rather harsh reaction conditions gave only a small amount of the desired *t*-Boc protected aglycone V. Probably due to steric hindrance these protection reactions were more difficult than for other glycopeptide antibiotics having a free amino group at position 15[12]. However, a benzylation with benzylbromide in the presence of a base (NEt_3) took place smoothly with quantitative yield of the corresponding *N*-benzyl derivative VI without the concomitant formation of a benzylester.

Esters of A-40926 Aglycone

Most of the esters could be synthesized directly using the corresponding alcohol under acidic catalysis. For the formation of the methyl- and ethyl ester VII and VIII the use of aqueous HCl at room temperature was sufficient. Esters of alcohols with longer alkyl chains or carrying a free hydroxyl group (IX ~ XI) required prolonged heating at +70°C with sulfuric acid in the corresponding alcohol (Table 1).

The preparation of halogenalkyl esters and other esters required first protecting the methylated amino group at position 15 to avoid its concomitant alkylation.

Starting from the *N*-benzylated aglycone VI, the free carboxyl group could be esterified easily and selectively with 1,3-dibromopropane and chloroacetonitrile to give the corresponding 3-bromo-propyl ester XII and cyanomethyl ester XIV, respectively.

Clean and smooth removal of the benzyl protecting group from XII to give XIII was carried out by hydrogenolysis at pH 2 in a methanol/water mixture using 10% Pd/C as a catalyst.

Table 2. *In vitro* antibacterial activity of derivatlves of A-40926 aglycone.

Organism	MIC (mcg/ml)										
	A-40926	Aglycone A-40926	I	II	III	IV	V	VI	VII	VIII	IX
Staphylococcus aureus L165	0.13	0.13	0.25	0.25	0.5	0.13	0.25	0.13	0.13	0.06	0.13
S. epidermidis ATCC 12228	0.13	0.13	0.5	0.25	0.5	0.13	0.13	0.06	0.13	0.06	0.13
S. haemolyticus L602	4	0.5	4	4	16	1	1	0.5	0.5	0.25	0.25
Streptococcus pyogenes L49	0.03	0.5	0.13	1	0.13	0.5	0.25	0.25	0.13	0.13	0.13
S. pneumoniae L44	0.06	0.25	0.5	2	1	0.25	1	0.5	0.25	0.13	0.25
E. faecalis ATCC 7080	0.13	0.25	1	2	4	1	1	0.5	0.25	0.13	0.13
Neisseria gonorrhoeae L997	0.5	16	64	4	128	32	64	64	64	16	32
Haemophilus influenzae ATCC 19418	32	32	128	64	128	64	>128	128	64	32	128
Escherichia coli L47	>128	128	n.d.	>128	>128	128	>128	>128	64	32	64

Organism	MIC (mcg/ml)										
	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX
Staphylococcus aureus L165	0.13	0.13	0.5	0.06	0.13	0.5	0.25	0.5	0.13	0.25	0.13
S. epidermidis ATCC 12228	0.13	0.13	0.13	0.06	0.06	0.06	0.13	0.13	0.13	0.25	0.13
S. haemolyticus L602	1	0.5	2	0.5	1	1	0.5	1	0.5	4	2
Streptococcus pyogenes L49	0.5	0.13	0.5	0.13	0.5	0.25	0.5	0.25	0.5	4	2
S. pneumoniae L44	0.5	0.5	0.5	0.13	0.5	1	1	1	1	2	1
E. faecalis ATCC 7080	0.5	0.25	2	0.13	0.5	1	0.5	0.5	0.5	1	0.5
Neisseria gonorrhoeae L997	4	32	>128	16	128	>128	64	128	64	128	32
Haemophilus influenzae ATCC 19418	128	128	>128	128	128	>128	128	>128	128	64	32
Escherichia coli L47	n.d.	64	>128	128	>128	>128	64	>128	128	>128	>128

Amides of A-40926 Aglycone

The synthesis of amides of A-40926 aglycone was accomplished through the activated ester method.

An efficient reagent to activate the carboxyl group was chloro-acetonitrile which quantitatively yielded the desired cyanomethyl ester XIV starting from the N 15-protected aglycone VI.

With this intermediate the N^{63}-amide XV, and the N^{63}-methylamide XVII were synthesized.

All amides could be deprotected using the conditions developed for the debenzylation of compound XII to give XVI and XVIII, respectively.

Halogenation of A-40926 Aglycone

Attempts to introduce additional halogen atoms, or to replace chlorine with bromine in glycopeptide antibiotics by fermentation in the presence of bromine salts, have been reported for aridicins[16] and actaplanin[17], respectively. For aridicins neither antibiotic production nor normal cell growth took place. Only with actaplanin was the substitution of bromine for one of the chlorines observed.

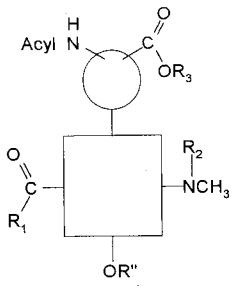
We attempted to manipulate the number and the type of halogen atoms present in the peptide backbone by chemical means.

Bromination of A-40926 aglycone with liquid bromine in DMF gave rise to two new, more lipophilic products, XIX and XX, which could be separated by prep. HPLC. NMR studies revealed that one product contained a bromine atom at position 41 (see Fig. 1). The other compound contained two bromine atoms (FAB: MH expected.: 1368.1, MH found: 1368.1). The exact position of the second atom could not be determined by spectroscopy and is still unknown.

Derivatives of A-40926

Starting from the complete molecule several derivatives were synthesized which are reported in Table 3. The reactivities of the carboxyl groups of the *N*-acylamino-glucuronic acid and to the peptide core are quite different. Reaction of A-40926 with primary alcohols and sulfuric acid cleanly and selectively esterified the carboxyl group of the sugar moiety. Double esterification was observed to a small extent only with excess reagents and long reaction times. Reaction with secondary alcohols such as *i*-propanol did not lead to esterification but to cleavage of the *N*-acylamino glucuronic acid moiety, producing the mannosyl aglycone (Fig. 1, R′=H).

Table 3. Derivatives A-40926.



Compound	R_1	R_2	R_3	R''	Yield (%)	Formula	MH (calc.)	MH (FAB, found)
XXI	OH	H	CH_3	Mannose	99	$C_{84}H_{90}Cl_2N_8O_{29}$	1745.6	1745.5
XXII	OH	H	C_2H_5	Mannose	49	$C_{85}H_{92}Cl_2N_8O_{29}$	1759.5	1759.6
XXIII	OH	H	C_3H_7(n)	Mannose	91	$C_{86}H_{94}Cl_2N_8O_{29}$	1773.6	1773.4
XXIV	OH	H	C_4H_9(n)	Mannose	66	$C_{87}H_{96}Cl_2N_8O_{29}$	1787.6	1787.6
XXV	OH	H	C_2H_4OH	Mannose	18[a]	$C_{85}H_{92}Cl_2N_8O_{30}$	1775.5	1775.4
XXVI	OH	H	C_4H_8OH	Mannose	55[a]	$C_{87}H_{96}Cl_2N_8O_{30}$	1825.5	1825.4
XXVII	OH	H	C_2H_4Br	Mannose	5[a]	$C_{85}H_{91}BrCl_2N_8O_{29}$	1837.5	1859.4[b]
XXVIII	OH	H	C_2H_4Br	H	9[a]	$C_{79}H_{81}BrCl_2N_8O_{24}$	1675.4	1697.4[b]
XXIX	OH	Bzl	H	Mannose	97	$C_{90}H_{94}Cl_2N_8O_{29}$	1821.6	1821.8
XXX	OCH_2CN	Bzl	CH_2CN	Mannose	93	$C_{94}H_{96}Cl_2N_{10}O_{29}$	1899.6	1899.5
XXXI	OH	H	CH_3	H	12[a]	$C_{78}H_{80}Cl_2N_8O_{24}$	1583.5	1583.5

[a] After preparation HPLC.
[b] $M^+ + Na$.

Some other mono-esters with free functionalities were synthesized by the same method. By dissolving A-40926 directly in 1,2-dihydroxyethane or 1,4-dihydroxybutane and adjusting the pH to 2 with sulfuric acid, the corresponding mono-2-hydroxyethyl and 4-hydroxybutyl esters, XXV and XXVI were isolated. With 2-bromoethanol (probably due to the presence of HBr) an esterification without the addition of the catalyst was possible. However, the reaction was not homogeneous; 4 products were formed simultaneously: A-40926 aglycone, mannosylaglycone, the desired 2-bromoethyl ester XXVII, and its demannosylated species XXVIII. It is noteworthy that all monoesters described could be produced only by reaction of A-40926 with neat alcohols under acidic catalysis. When the alcohols were diluted with other solvents (DMF *etc.*), no reaction took place.

Selective protection of the amino group of A-40926 at position 15 was carried out by reaction with benzylbromide in the presence of NEt_3 without concomitant esterification of the two carboxyl groups. Subsequently the two carboxyl groups of this N^{15}-protected derivative XXIX were esterified with bromoacetonitrile to form cleanly the cyanomethyl diester XXX.

Since demannosylated A-40926 had improved activity against coagulase-negative Staphylococci[11], a method for selective cleavage of this carbohydrate was developed (Scheme 1). Heating A-40926 in DMSO - 37% HCl (9 : 1, v/v) for 5 hours at +65°C resulted in the formation of a mixture of *N*-acylaminoglucuronyl aglycone complex and aglycone in the ratio 2 : 3, which could be separated by preparative HPLC. Prolonged reaction time and higher temperature caused cleavage of all of the sugars, leading to the aglycone. We tested whether selective cleavage was possible starting from a derivative of A-40926. For this purpose the monomethyl ester of A-40926 (compound XXI), which had antimicrobial activity similar to that of A-40926 (Table 4) was chosen.

We found that selective cleavage of the mannose from this compound was feasible. In order to improve the yield of the desired *N*-acylaminoglucuronyl aglycone derivative the hydrolysis conditions were modified. By changing the solvent/hydrochloric acid ratio from 9 : 1 to 78 : 1, lowering the reaction temperature to below +60°C and increasing the reaction time (7 days) the ratio of the desired *N*-acyl-aminoglucuronyl aglycone derivative XXXI to the undesired aglycone was improved from 2 : 3 to 1.4 : 1.0.

All derivatives were checked for homogeneity and purity by reversed phase HPLC. FAB-MS and NMR analyses confirmed all the presumed structures. The FAB-MS values are listed in Tables 1 and 3 for the derivatives of A-40926 aglycone and A-40926, respec-

Table 4. *In vitro* antibacterial activity of derivatives of A-40926.

Organism	MIC (mcg/ml)												
	A-40926 complex	Aglycone A-40926	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII	XXVIII	XIX	XXX	XXXI
Staphylococcus aureus L165	0.13	0.13	0.13	0.13	0.5	0.25	0.13	0.25	0.13	0.13	0.25	1	0.06
S. epidermidis ATCC 12228	0.13	0.13	0.25	0.25	2	0.13	1	2	0.25	0.13	0.25	0.25	0.06
S. haemolyticus L602	4	0.5	4	8	16	16	8	16	4	0.5	4	4	0.5
Streptococcus pyogenes L49	0.03	0.5	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.13	0.06
S. pneumoniae L44	0.06	0.25	0.06	0.06	0.25	0.13	0.06	0.06	0.13	0.13	0.06	0.13	0.06
E. faecalis ATCC 7080	0.13	0.25	0.13	0.25	2	0.5	0.13	0.13	0.25	0.25	1	1	0.13
Neisseria gonorrhoeae L997	0.5	16	0.5	2	16	8	0.25	16	1	4	4	8	2
Haemophilus influenzae ATCC 19418	32	32	64	64	64	64	64	64	64	32	>128	>128	16
Escherichia coli L47	>128	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128

Table 5. Activity of derivatives of A-40926 aglycone and A-40926 against *Streptococcus pyogenes*. Septicemia in the mouse.

Compound	ED_{50} (mg/kg)
A-40926 aglycone	6.2
Aglycone ethyl ester (VIII)	10
A-40926	0.41
Monomethyl ester (XXI)	0.18
Monoethyl ester (XXII)	0.23
Mono-2-hydroxyethyl ester (XXV)	0.18
Mono-4-hydroxybutyl ester (XXVI)	0.41
Demannosyl-monomethyl ester (XXXI)	0.31
Demannosyl-mono-2-bromoethyl ester (XXVIII)	0.31

Table 6. Main ^1H NMR data (cm^{-1}) of A-40926[10] (A) and its aglycone[11] (a).

Proton	A	a	Proton	A	a
x1	5.37	5.36	6b	7.64	7.75
x2	4.84	4.93	6e	7.19	7.20
x3	6.09	6.05	6f	7.39	6.38
x4	5.49	5.54	7d	6.75	6.26
x5	4.41	4.34	7f	6.44	6.26
x6	4.08	4.09	NCH_3	2.45	2.50
x7	4.50	4.43	w1	n.d.	9.21
z2	2.88	2.89	w2	8.14	8.02
z*2	3.32	3.33	w3	7.46	7.57
z6	5.06	5.09	w4	7.41	7.20
1b	6.67	6.76	w5	8.37	8.38
1e	6.96	7.01	w6	6.65	6.61
1f	7.12	7.16	w7	8.50	8.37
2b	7.07	7.09	M1	5.31	
2c	7.03	7.12	G1	5.36	
2e	6.93	6.92	G2	3.74	
2f	7.74	7.73	G2NH	7.67	
3d	6.71	6.60	G3	3.62	
3f	6.39	6.50	F1	2.00	
4b	5.76	5.76	F2	1.40	
4f	5.04	5.04	F3	1.05	
5b	7.06	7.08	~F8	~1.25	
5e	6.71	6.66	F9	1.40	
5f	6.71	6.73	F10	0.80	

The proton nomenclature adopted is that of Fig. 1.

tively. NMR assignments of A-40926 and of A-40926 aglycone are reported in Table 6, NMR assignments of A-40926 derivatives I~XXXI are reported in Table 7.

Experimental

Evaporation of solvents was carried out, after addition of *n*-butanol to prevent foaming, with a rotary evaporator at 45°C under vacuum. If not otherwise stated the final products were washed with diethyl ether and dried at 40°C under vacuum. Separations were performed using silanized Silica gel 60 (0.06~0.2 mm) or Silica gel RP-8 (LiChroprep 40~63 μm; Merck) with normal columns or with preparation HPLC using a Waters model 590 equipped with a 481 UV detector and a LiChrosorb RP-18 preparation column, 10 μm (size 250×50 mm, loop 5 ml, flow rate 30 ml/minute). HPLC was also used to monitor reactions, chromatographic fractions and purity of the compounds using a Varian 5000 apparatus equipped with a 2050 UV-detector at 254 nm and LiChrosorb RP-8, 5 μm or RP-18, 10 μm columns (125×4 and 250×4 mm, respectively). Injection volume: 10 μl; flow rate: 1.5 ml/minute; mobile phase, (A) 0.02 M aq NaH_2PO_4, (B) CH_3CN.

The linear step gradient was programmed as follows:

Time (minutes):	0	10	15	20	25	30	35
% CH_3CN in A:	15	23	27	31	35	55	15

All the compounds were characterized with FAB-MS (Table 3) and NMR spectra (Tables 6 and 7) and the results were in accordance with the proposed structure. In Table 6 complete assignments of A-40926 and its aglycone are reported. Table 7 reports those NMR assignments that are diagnostic for the chemical modification that was introduced in the parent molecule.

The ^1H NMR 1D and 2D spectra (COSY phase

Table 7. Main ^1H NMR spectral differences of A-40926 and its aglycone derivatives as compared with their parent compounds.

Derivative		Introduced chemical groups and their chemical shifts (ppm)
I	a	NCH_3: 2.88; $CH_3C=O$: 2.08
II	a	NCH_3: 2.69; CH=CH: 6.23, 5.9
III	a	NCH_3: 2.45
IV	a	$N(CH_3)_2$: 2.40
V	a	$(CH_3)_3C-O$: 1.45,1.50: NCH_3: 2.76
VI	a	C_6H_5: 7.30; CH_2: 3.76; CH_3: 2.31
VII	a	$COOCH_3$: 3.69
VIII	a	$COOCH_2$: 4.10; CH_3: 1.30
IX	a	$COOCH_2$: 4.10; $(CH_2)CH_2$: 1.60; CH_3: 0.86
X	a	$COOCH_2$: 4.10, $CH_2(OH)$: 3.57
XI	a	$COOCH_2$: 4.09; $(COOCH_2)CH_2$: 1.63; $CH_2(CH_2OH)$: 1.45; CH_2OH: 3.67
XII	a	$COOCH_2$: 4.31; $(COOCH_2)CH_2$: 2.12; CH_2Br: 3.48; NCH_3: 2.31; NCH_2: 3.75; C_6H_5: 7.35
XIII	a	$COOCH_2$: 4.28; $(COOCH_2)CH_2$: 2.13; CH_2Br: 3.49
XIV	a	$COOCH_2CN$: 5.09; NCH_3: 2.31; NCH_2: 3.76; C_6H_5: 7.38
XV	a	$CONH_2$: 6.70; NCH_3: 2.31; NCH_2: 3.76; C_6H_5: 7.35
XVI	a	$CONH_2$: 6.70
XVII	a	$CONHCH_3$: 2.67; NCH_3: 2.33; NCH_2: 3.78; C_6H_5: 7.30
XVIII	a	$(CONH)CH_3$: 2.62
XIX	a	The chemical shift of 7f (6.50 ppm) proton is in agreement with Br attached to 7b position
XX		
XXI	A	$COOCH_3$: 3.63
XXII	A	$COOCH_2$: 4.10; $(COOCH_2)CH_3$: 1.35
XXIII	A	$COOCH_2$: 4.05; $(COOCH_2)CH_2$: 1.6; $(CH_2)CH_3$: 0.82
XXIV	A	$COOCH_2$: 4.30; $(CH_2)CH_2(CH_2)$: 1.60; CH_3: 1.10
XXV	A	$COOCH_2$: 4.10; $CH_2(OH)$: 3.75
XXVI	A	$COOCH_2$: 4.25; $(COOCH_2)CH_2$: 1.40; $COOCH_2CH_2)CH_2$: 1.57; $CH_2(OH)$: 3.73
XXVII	A	$COOCH_2$: 4.32; CH_2Br: 3.67
XXVIII	A	$COOCH_2$: 4.32; CH_2Br: 3.70
XXIX	A	$N-CH_3$: 2.30; $C_6H_5(CH_2)$: 7.30; $(C_6H_5)CH_2$: 3.78
XXX	A	NCH_3: 2.30; $C_6H_5(CH_2)$: 7.30; $(C_6H_5)CH_2$: 3.72; CH_2CN: 4.90
XXXI	DM	$COOCH_3$: 3.62

Experimental conditions: The ^1H NMR 1D and 2D spectra (COSY phase sensitive double quantum filter) were performed with a Bruker instrument at 500 MHz in DMSO-d_6 solution at 30°C.

A=A-40926 derivative, a=A-40926 aglycone derivative, DA=demannosyl A-40926 derivative).

sensitive double quantum filter) were performed with a Bruker AM instrument at 500 MHz in DMSO-d_6 solution at 30°C. In the Table 7 the assignments are reported for the groups chemically introduced into A-40926 (A), its aglycone (a) or its demannosyl pseudo-aglycone (DA). In the various derivatives the overall pattern of the spectra is unchanged with respect to that of the parent compound while changes of chemical shifts concerning some specific part of the molecule add evidence to the derivative structure. In fact, the presence of an amide on amino acid 7 exerts a downfield shift on z6 proton (about 0.15 ppm) as already observed in the field of teicoplanin family[22]. The presence of an ester on amino acid 7 causes an inductive effect (downfield shift) on x7 (about 0.06 ppm) and an anisotropic effect (upfield shift) on 7f (about 0.15 ppm). An analogous effect was also already found in the field of teicoplanin family[23].

FAB-MS positive ion spectra were obtained on a Kratos MS-50 focusing mass spectrometer of 3000 dalton mass range, using 8 kV accelerating voltage. The instrument was operating under computer control. To obtain high quality data, a DS-9O data system in "raw data" acquisition was used. For mass calibration a mixture of CsI and NaI was used. For FAB, a saddle field atom gun was used with Xe gas (2×10^{-5} torr pressure) at 6 kV voltage and 1 mA current. The samples were dissolved in methanol or DMF depending on solubility. 1 μl of this solution was mixed with 1 μl of thioglycerol matrix containing 0.1 μl CH_3COOH on the target.

A-40926 Aglycone

To a stirred solution of 5 g (2.89 mmol) A-40926 in 80 ml DMSO, 4.5 ml of conc. HCl were added and the

mixture heated for 9.5 hours at +80°C. Then the solution was dripped into 100 ml of butanol-ether (1:1), the supernatant decanted and the remaining brown liquid suspended twice in 100 ml of the butanol-ether mixture. The obtained semi-solid material was dissolved in 25 ml butanol and precipitated with 100 ml ether. The solid product was collected by filtration. For purification the material was dissolved in a mixture of 30 ml H_2O and 0.6 ml CH_3CN and loaded on a column (i.d. 4 cm, length 6.5 cm) filled with 40 g silanized SiO_2. The column was washed with water (*ca.* 1 liter), then developed with 1.5 liters of a 0~20% gradient of CH_3CN in H_2O and finally eluted isocratically with 2 liters of 20% CH_3CN-H_2O. The fractions (20 ml each) containing the aglycone were pooled and evaporated to a small volume after addition of butanol. The solid which separated after addition of ether was collected, yielding 2.35 g (85.5%) of A-40926 aglycone in a fairly pure state.

N^{15}-Acetyl-A-40926 Aglycone (I)

To a solution of 250 mg (0.21 mmol) aglycone in 15 ml DMF were added 0.3 ml (2.2 mmol) NEt_3, bringing the pH to 9. The mixture was cooled to −20°C and 20 µl (0.21 mmol) acetic anhydride were added dropwise with vigorous stirring. 0.5 hours later 100 ml of ether were added yielding a solid which was collected and washed thoroughly with ether. Yield: 258 mg (99.7%).

N^{15}-(3-Carboxypropenyl)-A-40926 Aglycone (II)

335 mg (0.276 mmol) aglycone were dissolved in 14 ml dry DMF and the pH adjusted to 9 with 0.2 ml NEt_3 (1.44 mmol). 54 mg (0.552 mmol) maleic acid anhydride were added with stirring at room temperature. 1.5 hours later most of the solvent was evaporated. Upon adding ether a brownish solid precipitated which could be collected by filtration yielding 319 mg (88.1%) of the title compound.

N^{15}-Sulfo-A-40926 Aglycone (III)

A solution of 250 mg (0.21 mmol) aglycone in 15 ml DMF was adjusted to pH 10 with 0.6 ml NEt_3 (4.3 mmol). The mixture was cooled to −20°C and 0.14 ml (2.1 mmol) chlorosulfonic acid dissolved in 0.85 ml DMF were added with stirring. After 10 minutes ether was added and the precipitate collected by filtration, washed several times with ether, and lyophilized yielding 267 mg (100%) of the title compound.

N^{15}-Methyl-A-40926 Aglycone (IV)

To a suspension of 200 mg (0.165 mmol) aglycone in 50 ml H_2O and 6 ml DMF were added 0.5 ml of 40% aqueous formaldehyde solution followed by 13.9 mg (0.165 mmol) $NaHCO_3$. With vigorous stirring at room temperature 50 mg (0.8 mmol) sodium cyanoborohydride were added; the milky suspension cleared. 10 minutes later the pH was adjusted to 4.5 with acetic acid, the solution was diluted with 150 ml water and the aqueous phase was extracted three times with butanol. The combined butanolic phases were washed with water and the organic phase was reduced to a small volume. Upon adding ether, 53.0 mg (26.2%) of *N,N*-dimethyl-aglycone were obtained.

N^{15}-*tert*-BOC-A-40926 Aglycone (V)

To a solution of 400 mg (0.33 mmol) aglycone in 80 ml DMF we added 135 mg (0.452 mmol) 2,4,5-trichlorophenyl-*tert*-butylcarbonate and 58 µl (0.42 mmol) TEA. The mixture was heated to 60°C with stirring for 6 hours, and an additional 135 mg 2,4,5-trichlorophenyl-*tert*-butylcarbonate (0.452 mmol) were added. After 22 hours, 270 mg (0.904 mmol) 2,4,5-trichlorophenyl-*tert*-butylcarbonate and 116 µl (0.84 mmol) TEA were added. The temperature was maintained at 60°C for 51 hours and then most of the solvent was removed. After addition of *ca.* 20 ml butanol, ether was added and the precipitate collected and purified on a column filled with 20 g silanized SiO_2 and equilibrated with 20% CH_3CN in water. The column was developed with 1 liter of a 20~50% gradient of CH_3CN and then isocratically with 0.5 liter of 50% CH_3CN-H_2O. The title compound was present in fractions 30~45, which were pooled and evaporated to dryness with butanol. Yield: 62 mg (14.4%).

N^{15}-Benzyl A-40926 Aglycone (VI)

To a solution of 200 mg (0.165 mmol) A-40926 aglycone in 40 ml abs. DMF we added 25.3 µl (0.18 mmol) triethylamine and 12 µl (0.198 mmol) benzyl bromide; the mixture was stirred for 22 hours at room temperature. The volume of solvent was then reduced to *ca.* 10 ml. Upon addition of ether, a precipitate appeared which was purified on a column containing 20 g silanized SiO_2 equilibrated with 10% acetonitrile. The column was developed with 1 liter of a 10~40% acetonitrile gradient. Fractions of about 10 ml were collected and those containing the title compound (35~100) were pooled and reduced to a small volume. Upon adding ether, 108 mg (50.3%) of the *N*-benzylated aglycone were collected.

A-40926 Aglycone Methyl Ester (VII)

100 mg (0.082 mmol) aglycone were dissolved in 10 ml methanolic HCl (10%) and left at room temperature for *ca.* 18 hours. After concentration to a smaller volume ether was added. The precipitate was collected by filtration and triturated thoroughly with ether yielding 74 mg (73%) of a whitish powder.

A-40926 Aglycone Ethyl Ester (VIII)

200 mg (0.164 mmol) aglycone were added to 20 ml of ethanol containing 20% (w/w) gaseous HCl and stirred overnight at room temperature (18 hours). The solution was then concentrated, ether was added and the precipitate was collected. The crude material was dissolved in acetonitrile-water and then loaded onto a column containing 20 g silanized Silica gel equilibrated with 10%

CH_3CN in water. The column was developed with 1.5 liters of a linear gradient of 10~30% acetonitrile in water. The fractions (10 ml each) containing the ethyl ester were combined, butanol was added and the solution was evaporated to a smaller volume. Upon adding ether a solid separated consisting of 50 mg (25%) of the title compound.

A-40926 Aglycone Propyl Ester (IX)

A solution of 250 mg (0.206 mmol) aglycone in 30 ml *n*-propanol was adjusted to pH 2 with conc. H_2SO_4 and heated for 9 hours at 80°C. After filtration, most of the solvent was evaporated. Upon adding 10 ml water a solid separated (123 mg) which was collected by filtration. The filtrate was extracted twice with 30 ml butanol and the combined organic phases washed twice with water. The butanolic phase was then concentrated to a smaller volume and upon adding ether a second crop of the desired propyl ester was obtained (100 mg).

The two crops were combined and purified with preparation HPLC using 5 liters of 40% CH_3CN in 0.02 M NaH_2PO_4 buffer (pH=4.7) with a flow rate 40 ml/minute on a RP-18 column, 10 μm (Merck) (250 × 50 mm).

The fractions containing the desired compound were pooled and evaporated to a small volume and the aqueous phase was extracted twice with butanol. The organic extracts were combined and concentrated. Precipitation with ether gave 45 mg (17.4%) of very pure propyl ester.

A-40926 Aglycone-2-hydroxyethyl Ester (X)

200 mg A-40926 aglycone (0.164 mmol) were dissolved in 5 ml 1,2-ethanediol and the pH of the solution was adjusted to 2 with H_2SO_4. The solution was heated at +60°C for 17 hours and for 4 hours at +80°C, concentrated, diluted with a mixture of 19% CH_3CN in H_2O and injected directly onto a preparation column (RP-10, 10 μm; 250 × 50 mm) equilibrated with 19% CH_3CN in 0.02 M NaH_2PO_4 buffer. The column was developed isocratically at a flow rate of 40 ml/minute and the fractions assayed with HPLC. The fractions containing the title compound were pooled, the acetonitrile was evaporated and the aqueous phase was extracted three times with butanol (25 ml). The work-up yielded 66 mg (32%) of the title compound.

A-40926 Aglycone-4-hydroxybutyl Ester (XI)

To 5 ml of 1,4-butanediol 150 mg A-40926 aglycone (0.041 mmol) were added. The pH was adjusted to 2 with conc. sulfuric acid. The mixture was heated for 45 hours at 60°C, then diluted with water (100 ml) and the aqueous phase was extracted three times with 20 ml butanol. The organic layers were pooled, reduced to a small volume and poured into ether; the product precipitated in a semi-solid form. This material was redissolved in butanol and poured once more into ether yielding a whitish powder which was collected by filtration, triturated with ether and dried at 60°C. Yield: 93 mg (59%).

3-Bromopropyl Ester N^{15}-Benzyl-A-40926 Aglycone (XII)

0.86 ml (0.844 mmol) 1,3-dibromopropane were added with stirring to a solution of 500 mg (0.384 mmol) of *N*-benzylated aglycone VI and 53.6 μl (0.384 mmol) triethylamine in 20 ml DMF. The reaction mixture was heated at +60°C with stirring for 5 hours. An additional 43.1 μl (0.422 mmol) 1,3-dibromopropane and 26.8 μl (0.192 mmol) NEt_3 were added. After heating at +60°C for a further 3 hours the solution was poured into 200 ml of ether - *n*-hexane (3 : 1) and the precipitate collected by centrifugation. The supernatant was discarded and the residue resuspended twice with ether yielding 500 mg which was purified with preparation HPLC using 8 liters of 38% CH_3CN in 0.02 M sodium biphosphate buffer (isocratic, flow rate 40 ml/minute).

The fractions containing the desired product were pooled, most of the solvent was evaporated and the aqueous phase was extracted three times with *n*-butanol. Evaporation to dryness yielded 97 mg (18.3%) of the title compound.

A-40926 Aglycone-3-bromopropyl Ester (XIII)

50 mg (0.035 mmol) *N*-benzylated 3-bromopropyl ester XII were dissolved in a mixture of 5 ml CH_3OH and 0.5 ml H_2O and the pH was adjusted to 2 with 2 ml 0.1 N HCl. 3 mg Pd on carbon (10%) were added and the solution hydrogenated at ambient pressure. 4 mg fresh catalyst were added after 1.5 hours and 2.7 mg after an additional 4 hours. 7 hours later the reaction was complete, the catalyst was filtered off and washed carefully with methanol and the filtrate was reduced to a small volume. Ether was added, yielding a white precipitate which was collected by centrifugation. The supernatant was discarded and the residue was washed thoroughly with ether yielding 34 mg (73%) of the debenzylated ester as hydrochloride.

N^{15}-Benzyl-A-40926 Aglycone-cyanomethyl Ester (XIV)

1.3 g (1.0 mmol) of *N*-benzylated aglycone VI were dissolved in 100 ml DME and the pH of the solution was brought to 8 with 0.23 ml (2.0 mmol) triethylamine. After addition of 63.5 μl (0.998 mmol) chloroacetonitrile the mixture was stirred at room temperature for 6 hours. Then an additional 128 μl (2.0 mmol) of reagent were added, followed by 230 μl (2.0 mmol) TEA and 128 μl (2.0 mmol) chloroacetonitrile 3 hours later. After heating at +60°C for 4 hours the reaction was complete and the solvents were reduced to a smaller volume. Upon adding ether, 1.3 g (97%) of pure title compound were isolated.

N^{15}-Benzyl-A-40926 Aglycone Amide (XV)

100 mg (0.075 mmol) *N*-benzylated cyanomethyl ester XIV were added with stirring at 0~5°C in 4 ml of ethanol containing 13% (w/v) gaseous ammonia. After 19 hours an additional 1 ml of reagent was added and 7 hours later the reaction mixture was evaporated to dryness.

The residue was purified by preparative HPLC under isocratic conditions with 5 liters of 29% CH_3CN in 0.02 M sodium biphosphate buffer. Yield: 37 mg (38%).

A-40926 Aglycone Amide (XVI)

25 mg (0.0192 mmol) of *N*-benzylated amide XV were dissolved in a mixture of 3.5 ml H_2O and 0.2 ml 0.1 N HCl. 5 mg of 10% Pd on charcoal were added and hydrogenated at room temperature and ambient pressure. After 2 hours 5 mg fresh catalyst were added, followed by 1 mg 7 hours later. After 12 hours the reaction mixture was filtered, the residue washed with DMF and water and evaporated to dryness. Yield: 13.2 mg (58%) of the title compound.

N^{15}-Benzyl-A-40926 Aglycone-N^{63}-methylamide (XVII)

100 mg (0.075 mmol) *N*-benzylated cyanomethyl ester XIV were added with stirring at room temperature to 4 ml of 100 g methylamine in 300 ml abs. ethanol. After 1 hour the pH was adjusted to 4.5 with glacial acetic acid and the reaction mixture concentrated to a small volume. This was diluted with 50% acetonitrile in 0.02 M NaH_2PO_4 buffer and injected directly onto a preparation HPLC column (250 × 25 mm) using the same mixture for the separation (isocratic). The fractions containing the title compound were pooled and worked up as described above. Yield: 13 mg (13.3%).

A-40926 Aglycone-N^{63}-methylamide (XVIII)

10 mg (0.0076 mmol) of the *N*-benzylated-methylamide XVII were dissolved in a mixture of 1.5 ml CH_3OH and 0.3 ml H_2O. The pH was adjusted to 2 with 0.2 ml 0.1 N HCl, 3 mg 10% Pd on carbon were added and the mixture was hydrogenated under atmospheric pressure at room temperature. After 2.5 hours 5 mg fresh catalyst were added and 2 hours later 3 mg. 8.5 hours later the reaction was completed. The catalyst was filtered off and washed with methanol and the filtrate lyophilized after evaporation of the organic solvent. Yield: 3.2 mg (34.4%) of the title compound as hydrochloride.

41-Bromo-A-40926 Aglycone (XIX) and Di-bromo-A-40926 Aglycone (XX)

200 mg (0.165 mmol) aglycone were dissolved in 20 ml DMF and cooled in an ice-bath. A solution of 10 μl bromine (0.98 mmol) in 0.3 ml DMF was added dropwise. 20 hours later an additional 6.6 μl bromine (0.65 mmol) dissolved in 0.2 ml DMF were added, maintaining the temperature at 0 ~ 5°C. After 45 hours, 15 μl bromine (1.47 mmol) in 0.15 ml DMF were added. 5 hours later the reaction mixture was reduced to a small volume. Upon adding ether a solid separated which was filtered off and separated with preparation HPLC using 5 liters of 17.5% CH_3CN in 0.02 M NaH_2PO_4 (isocratic). Two fractions were collected and the product isolated. Yield: 23.2 mg (10.9%) mono-bromo aglycone; 77.5 mg (34.3%) di-bromo aglycone.

6^B-Methoxycarbonyl-A-40926 (XXI)

150 mg A-40926 (0.0866 mmol) were dissolved in 20 ml abs. methanol and the pH adjusted to 2 with conc. sulfuric acid. The mixture was stirred at room temperature for 26 hours. When the pH was brought to 6 with 0.15 ml NEt_3 a precipitate appeared. After addition of ether the precipitate was collected, washed thoroughly with ether and dried. Yield: 15 mg (99%).

6^B-Ethoxycarbonyl-A-40926 (XXII)

To a stirred solution of 250 mg (0.144 mmol) A-40926 in 30 ml abs. ethanol at room temperature we added 2 drops of concentrated sulfuric acid, bringing the pH to 3. 23 hours later the pH was brought to 7 with NEt_3. Upon adding ether a precipitate appeared which was collected by filtration. This precipitate was suspended in water (130 ml) and the aqueous phase was extracted twice with butanol. The extracts were combined and evaporated to a small volume. Upon addition of ether a solid precipitate was collected yielding 125 mg (49.2%).

6^B-Propoxycarbonyl-A-40926 (XXIII)

200 mg A-40926 (0.115 mmol) were dissolved in 50 ml abs. *n*-propanol. The pH was adjusted to 3 with 40 μl conc. H_2SO_4 and the solution was stirred at room temperature for 15 hours. The pH was adjusted to 7 with NH_4OH and the product was precipitated by adding ether. Yield: 267 mg (91.2%).

6^B-Butoxycarbonyl-A-40926 (XXIV)

200 mg A-40926 (0.115 mmol) were dissolved in 100 ml abs. butanol. The pH was adjusted as above and the solution was left to stand for 3 days at room temperature. The pH was then raised to 5 with 40 μl NH_4OH (32%), 100 ml of water were added, the organic layer was separated and the aqueous phase was extracted twice with butanol. The butanol extracts were pooled and reduced to a small volume. Upon addition of ether 136 mg (65.9%) of the title compound were collected.

6^B-2-Hydroxyethoxycarbonyl-A-40926 (XXV)

150 mg A-40926 (0.0866 mmol) were dissolved in 20 ml ethyleneglycol with stirring at room temperature. The pH was adjusted to 2 with 40 μl conc. sulfuric acid and the mixture was left to stand for 3 days. The solution was reduced to *ca.* 5 ml and directly separated by preparation HPLC with 5 liters of 31% CH_3CN in 0.02 M NaH_2PO_4 (isocratic). The isolation procedure yielded 27 mg (18%) of very pure ester.

6^B-4-Hydroxybutoxycarbonyl-A-40926 (XXVI)

To a solution of 400 mg A-40926 (0.230 mmol) in 25 ml of 1,4-butanediol we added 60 μl conc. sulfuric acid with stirring at room temperature. The solution was left to stand for 55 hours and then diluted with 50 ml of butanol. Upon addition of ether a solid precipitated which was collected and purified by preparation HPLC using 5 liters of 32% CH_3CN in 0.02 M NaH_2PO_4. Using the isolation

procedure, 227 mg (54.5%) of the title compound were obtained.

6^B-(2-Bromoethyl Ester) of A-40926 (XXVII) and Its Demannosylated Analogue (XXVIII)

300 mg A-40926 (0.173 mmol) were dissolved with stirring in 5 ml 2-bromoethanol for 22 hours at 0°C. The temperature was raised to room temperature and stirring was continued for 15 hours. The starting material was completely transformed into two more lipophilic compounds accompanied by large amounts of aglycone and mannosylaglycone. The solution was cooled to 0°C. Upon adding ether a precipitate appeared which was collected (295 mg) and then separated using preparation HPLC (RP-18, 10 um), with 34% acetonitrile in 0.02 M NaH_2PO_4 buffer. Two fractions of 15 mg (4.7%) and 27 mg (9.3%) were isolated which corresponded to the 2-bromoethyl ester XXIX and its demannosylated derivative XXX.

N^{15}-Benzyl-A-40926 (XXIX)

1.5 g (0.87 mmol) A-40926 were dissolved in 100 ml dry DMF and the solution was cooled to 0°C. The pH was adjusted to 7 with 0.26 ml (1.83 mmol) NEt_3. 0.11 ml (0.96 mmol) benzylbromide were added with stirring and 7 hours later an additional 20.6 μl (0.145 mmol) benzylbromide were added. After 48 hours ether was added and the precipitate was collected, washed carefully with ether and dried. Yield: 1.53 g (96.7%).

6^B-Cyanomethyl Ester of N^{15}-Benzyl-A-40926 Cyanomethyl Ester (XXX)

9.6 μl (0.138 mmol) bromoacetonitrile and 38.4 μl (0.276 mmol) TEA were added with stirring at room temperature to a solution of 250 mg (0.138 mmol) *N*-benzylated-A-40926 XXXI in 12 ml DMF. 22 hours later 9.6 μl (0.138 mmol) bromoacetonitrile and 19.2 μl (0.276 mmol) TEA were added and 45 hours later a third portion of 19.2 μl (0.276 mmol) bromoacetonitrile and 19.2 μl (0.138 mmol) TEA. After 52 hours ether was added and the precipitate was collected, yielding 242 mg (92.8%) of the title compound.

6^B-Methyl Ester of O^{42}-Demannosyl-A-40926 (XXXI)

To a solution of 650 mg (0.086 mmol) A-40926 methylester XXIII in 7 ml DMSO we added 50 μl 37% HCl, bringing the pH to 2. The mixture was heated at 50°C for 24 hours. Then an additional 25 μl HCl were added and after 48 hours further 15 μl thus maintaining the pH at 2.

7 days later HPLC showed the disappearance of A-40926 and a 1.4 : 1.0 ratio of *N*-acylaminoglucuronyl aglycone complex to aglycone. The reaction mixture was poured into a mixture of 15 ml ether and 5 ml butanol, yielding a solid precipitate (653 mg) which was collected by filtration and separated with preparation HPLC using isocratic conditions with 30% CH_3CN - 70% 0.02 M NaH_2PO_4 (5 liters), then 33% CH_3CN - 67% 0.02 M NaH_2PO_4 (2 liters), and finally 40% CH_3CN - 60% 0.02 M NaH_2PO_4 (2 liters). The fractions containing the desired compound were pooled, reduced to a smaller volume and extracted three times with butanol; the butanolic phase was reduced to a small volume. Upon addition of ether 68 mg (11.5%) of the title compound was collected.

In Vitro Antimicrobial Activity

MICs for most organisms were determined by microbroth dilution methodology. Inocula were approximately 10^4 cfu/ml. Incubation times were 18~24 hours, except for: *N. gonorrhoeae*, and *Haemophilus influenzae* (48 hours). All organisms were incubated at 37°C. *N. gonorrhoeae* and *H. influenzae* were incubated in a 5% CO_2 atmosphere. Media used were: Oxoid Iso-sensitest broth (staphylococci, *Enterococcus faecalis*, *Escherichia coli*); Difco Todd-Hewitt broth (streptococci); Difco GC base broth with 1% BBL IsoVitaleX for *N. gonorrhoeae*; Difco Brain-Heart Infusion broth with 1% Difco Supplement C for *H. influenzae*.

Experimental Septicemia in the Mouse

Control and treated groups contained five CD-1 mice (Charles River) weighing 18~22 g. They were infected ip with 0.5 ml of a bacterial suspension prepared by diluting an overnight culture of *Streptococcus pyogenes* C203 with sterile peptonized saline. Inocula were adjusted so that untreated animals died of septicemia within 48 hours. Antibiotics were administered sc immediately after infection. On the 7th day, the ED_{50} in mg/kg was calculated by the Spearman and Kaerber method[19] from the percentages of surviving animals at each dose.

Results and Discussion of Antibacterial Activity

The *in vitro* antibacterial activities (MICs) of the compounds synthesized starting from the A-40926 aglycone are reported in Table 2. The MICs of the compounds synthesized from A-40926 are in Table 4.

Aglycone Derivatives

None of the derivatives of A-40926 aglycone was more active than the parent compound against *N. gonorrhoeae*. However, some of the esters of A-40926 aglycone had interesting activity against other species. In particular, the ethylester VIII was more active than A-40926 against *Staphylococcus haemolyticus* (MIC 0.25 *vs.* 4 μg/ml) and also showed some activity against *Escherichia coli* (32 μg/ml). Neither the aglycone nor A-40926 was active against *E. coli*.

The 3-bromopropyl ester XII was inactive against *N. gonorrhoeae*. When the blocked amino group was deprotected by hydrogenolysis (to produce compound XIII) the MIC was lowered to 16 μg/ml; the activity against Gram-positive bacteria also improved. It is known that the free terminal amino group of glycopeptide antibiotics is important for their binding to the

carboxylate of the D-ala-D-ala terminus of nascent peptidoglycan in the bacterial cell wall.[20~22] Therefore, the fact that a free amino group conferred better antibacterial activity was not unexpected. A similar phenomenon was observed for some Gram-positive strains after deblocking the benzylated amino group of the amides XV and VII (to generate compounds XVI and XVIII), although the extent of improvement in antibacterial activity was less than that observed for the amides of teicoplanin.[5]

Derivatives with a modified charge on the amino group (*e.g.* III) did not show improved antibacterial activity. The introduction of additional halogen atoms (mono- and dibrominated compounds XIX and XX) led to a reduction in antibacterial activity.

The ethylester VIII, which was the most active of the aglycone derivatives *in vitro* was tested for activity against *S. pyogenes* septicemia in mice (Table 5), but it showed no activity at the highest dosage tested (10 mg/kg).

Derivatives of A-40926

None of the derivatives of A-40926 had better *in vitro* antibacterial activity than that of the parent compound. The methylester (XXI) and the 2-hydroxyethyl ester (XXV) had antibacterial activities similar to that of A-40926.

As expected from previous reports[11] the two demannosyl derivatives (XXVIII and XXXI) had better activity against coagulase-negative staphylococci (particularly *S. haemolyticus*) than the parent compounds XXVII and XXI (Table 4).

Some of the esters of A-40926 were also tested against *S. pyogenes* septicemia in mice (Table 5). The methyl ester XXI and the 2-hydroxyethyl ester XXV were more active than A-40926.

In conclusion, none of the derivatives of A-40926 or its aglycone had significantly better *in vitro* activity against Gram-positive bacteria or *N. gonorrhoeae*. However, the monomethyl and 2-hydroxyethyl esters (XXI and XXV) of A-40926 merit further study because of their better *in vivo* efficacy.

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