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Synthesis and antibacterial activity of alkyl derivatives of the glycopeptide antibiotic A40926 and their amides☆

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Abstract—New derivatives of the glycopeptide antibiotic A40926 were synthesized and evaluated for antimicrobial activity against VRE. Deacylated A40926 was obtained by microbial transformation of the parent antibiotic with the use of *Actinoplanes teichomyceticus* ATCC 31121. Regioselective synthesis of alkylated derivatives of Deacyl A40926 was carried out using lipophilic aliphatic and aromatic halides or aldehydes. Further modification of the two carboxylic acids was performed to increase antibiotic activity. Poor antimicrobial activity was observed for the derivatives obtained by lipophilic mono- or dialkylation of the amino groups present on the molecule, while simultaneous condensation of both carboxylic groups, in hydrophobic derivatives, with dibasic amines led to a strong increase in antibiotic activity.

1. Introduction

Recent chemical modification of glycopeptide antibiotics has been directed toward the synthesis of derivatives active against vancomycin-resistant enterococci (VRE), while retaining activity against susceptible Gram-positive bacteria including methicillin-resistant and coagulase-negative staphylococci. This effort resulted in the discovery of compounds with enhanced activity against VRE produced by reductive alkylation of vancomycin,[1] eremomycin,[2] and teicoplanin.[3] Among these new compounds, oritavancin[4] (Ly333328), derived from chloro eremomycin, is currently under clinical development.

Recent studies have shown that hydrophobic substituents play a major role in the antibacterial activity against VRE. Specific hydrophobic derivatives of eremomycin and vancomycin demonstrate that antibacterial activity against resistant strains is not based on binding to D-Ala-D-Lac. This activity appears due to inhibition of the transglycosylation step of bacterial peptidoglycan biosynthesis.[5]

The aim of this work was to take advantage of a previous description and to investigate if new mono- and dialkylated derivatives of Deacyl A40926 (DA40, see Fig. 1) could have an improved antibiotic activity against Van-A enterococci. Encouraging preliminary results were obtained in a previous study carried out on this molecule in which a slightly improved antimicrobial activity against Van-A enterococci was observed for an alkyl derivative of the DA40 glycopeptide.[6]

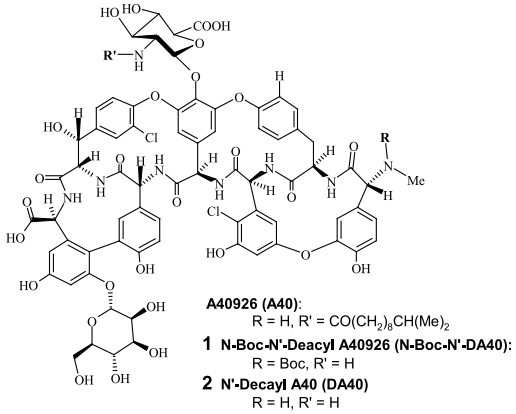

Figure 1. Structures of A40926, *N*-Boc-*N*′-deacylA40926, and Deacyl A40926.

Keywords: Glycopeptides; VRE; Antibiotic.
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This program started with from *N*-Boc-*N*′-DA40[7] (**1**) obtained from a recently described microbial deacylation of the Boc-protected natural scaffold.[8]

At the beginning of the project, selective N′-alkylation of DA40 was easily obtained by treatment of **1** with the desired aliphatic or aromatic aldehyde in the presence of $NaCNBH_3$ as a reducing agent, in THF/H_2O 1:1 as a solvent (see Chart 1).

To obtain N-alkylated isomers, we also studied the reductive alkylation on the deprotected DA40 (**2**) obtained from **1** by Boc removal with TFA at rt. We subjected **2** to the same reductive alkylation conditions described above. The sugar amine was again selectively alkylated, showing it to be the most reactive in this type of reaction. When a large excess (10–20 eqiuv) of aldehyde was used, second alkylation was observed on the same sugar amine, obtaining N′,N′-dialkylated derivatives, while the variation of the conditions (e.g., solvents, temperature or other reducing agents: $NaBH_4$ or $Na(AcO)_3BH$) did not lead to any desired alkylation of the peptidic N-terminal amino group.

The peptidic amine was selectively alkylated by reaction with 1–2 equivalents of primary alkyl bromides in DMF or DMSO. Alkylation on the sugar amine was observed only in the presence of a large excess of alkyl bromide. Depending on the nature of the alkyl bromide, carboxylic acids did not react when the reaction was carried out with pH in the range 6–8.

Even if the N,N′-dialkylated derivative could be obtained by further reductive alkylation of the N-mono-

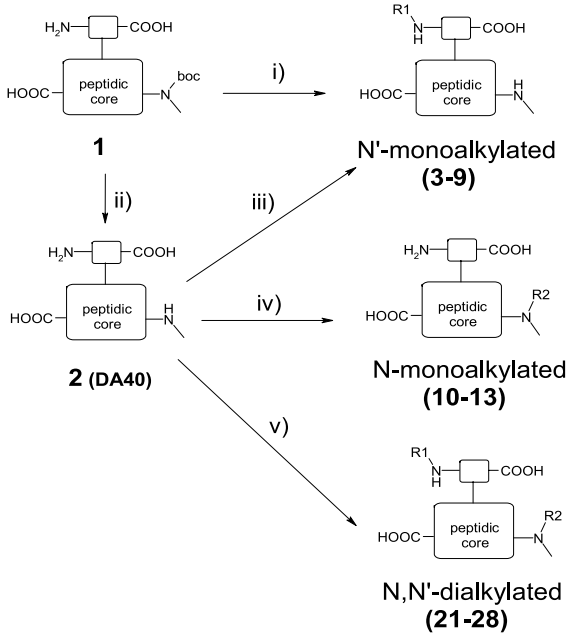


Chart 1. Reagents and conditions: (i) 1. RCHO, $NaCNBH_3$, THF/H_2O (1:1), rt 8 h; 2. TFA/CH_2Cl_2 (1:1), rt 15 min; (ii) TFA/CH_2Cl_2 (1:1), rt 15 min; (iii) RCHO, $NaCNBH_3$, THF/H_2O (1:1), rt 8 h; (iv) RCH_2Br, DMF or DMSO, pH 6–8, with TEA, rt 16 h; (v) 1. RCHO, $Me_3SiNHCOMe$, TEA in DMF 50 °C 15 min; 2. $Na(OAc)_3BH$, rt 2 h.

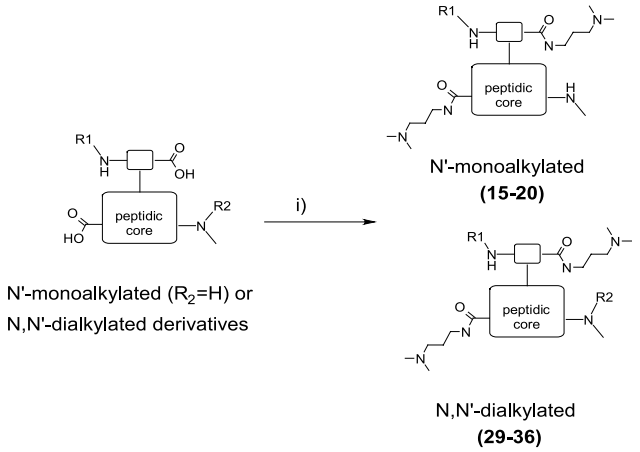


Chart 2. Reagents and conditions: (i) $Me_2(CH_2)_3NH_2$, PyBOP, in DMSO, pH 7.5–8, with TEA, rt 2 h.

alkylated derivative, we preferred to use a different approach to produce dialkylated derivatives. Preliminary activation of the N-terminal peptide amino group of DA40 (**2**) was achieved by nitrogen silylation with the use of trimethylsilyl-acetamide.[9] Following this procedure, **2** was reacted for 15 min at 50 °C with the desired aldehyde (5 mmol), of $Me_3SiNHCOMe$ (10 mmol), and TEA (2 mmol) in DMF and then $Na(OAc)_3BH$ was added and the reaction was stirred for 2 h at rt.

Mono- and dialkylated derivatives described above were transformed into the corresponding basic amides by condensation with $Me_2N(CH_2)_3NH_2$ in the presence of PyBOP as a condensing agent in DMF or DMSO at pH 7.5–8 (see Chart 2). All the compounds were tested for the antimicrobial activity against an appropriate panel of microorganisms. Tests were performed in microtiter plates following the NCCLS procedure.[10]

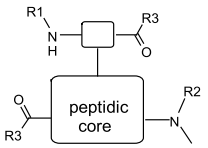
2. Results

A40 and DA40 (**2**) have no activity against VRE but have a range of 0.25–16 mg/L of activity against the other tested microorganisms (Table 1).

N- and N′-monoalkylated compounds (Table 1, **3–13**) maintained good or moderate activity against staphylococci, streptococci,[11] and Van-S enterococci (VSE), while only a few compounds showed marginal activity (Table 1, **3–6**) against VRE. All these activities were negatively affected by the presence of serum.

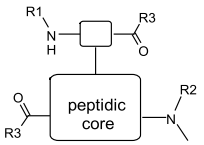
Further derivatization of the two carboxylic moieties, present on the DA40 alkyl derivatives, by amidation with alkyl or aryl amines resulted in derivatives having an antibacterial profile very much similar to that of the acidic derivatives of Table 1, while the corresponding basic diamides, produced by condensation with diamine $Me_2N(CH_2)_3NH_2$, proved to have interesting activity against VRE and showed only a moderate inactivation in the presence of serum (Table 1, **15–20**). In this class of basic amides, compounds **18** and **19** gave

Table 1. Monoalkylated derivatives

ID				MIC or MIC range (mg/L)								
				S. aureus		*S. epidermidis*		*S. haemolyticus*	*E. faecalis*		*E. faecium*	
	R_1	R_2	R_3	Met-S (1)	Met-R (2)	Met-S (1)	Met-R (1)	(1)	Van-S (3)	Van-A (4)	Van-S (1)	Van-A (4)
A40	Natural fatty acid	H	OH	0.25 [4]	0.5	4	nt	16	0.125 (1)	>64 [nt]	0.5	>64
2	H	H	OH	16 [nt]	16	nt	nt	nt	8–4 (2)	>128 [nt]	8	>128
3	4-*n*-Decyl-Ph-CH_2–	H	OH	4 [16]	2	32	nt	32	⩽0.125–0.25 (2)	4–1 (2) [>128]	0.5	2–64 (2)
4	4-OctyloxyPh-CH_2–	H	OH	2 [4]	1	16	nt	16	⩽0.125–0.25 (2)	8–64 (2) [>128]	0.5	32–>128 (2)
5	4-HexylOPh-CH_2–	H	OH	0.5 [16]	0.25	8	nt	nt	⩽0.125 (2)	16–64 (2) [>128]	0.25	64–>128 (2)
6	4-*n*-BuPhCH_2–	H	OH	0.25 [8]	0.25	0.25	4	16	⩽0.125	64–128 [>128]	2	64–>128
7	4-*n*-BuOPhCH_2–	H	OH	1 [8]	1	1	16	32	0.5–1	128–>128	1	64–>128
8	Naphthyl-2-CH_2–	H	OH	2 [8]	1	2	16	32	0.5–1	128–>128	2	128–>128
9	*n*-HexylCH(*n*-Bu)-CH_2–	H	OH	4 [32]	2	2	32	32	⩽0.125–0.25	>128	1	128–>128
10	H	4-*n*-BuOPhCH_2–	OH	4 [16]	2	32	128	>128	1–2	128–>128	2	64–>128
11	H	4-Ph-PhCH_2–	OH	1 [32]	1	2	16	32	0.25–0.5	128–>128	0.5	4–>128
12	H	Naphthyl-2-CH_2	OH	2 [16]	2	16	32	128	0.5–1	>128	1	64–>128
13	H	4-*n*-BuPhCH_2–	OH	1 [8]	1	1	8	32	0.25–0.5	128–>128	0.5	4–>128
14	H	H	$NH(CH_2)_3NMe_2$	1 [1]	2	1	nt	0.5	1 (2)	128–>128 (2)	1	>128
15	4-*n*-Decyl-Ph-CH_2–	H	$NH(CH_2)_3NMe_2$	2 [8]	2	1	nt	2	0.25	4 (2) [>32]	0.25	4–8 (2)
16	4-OctyloxyPh-CH_2–	H	$NH(CH_2)_3NMe_2$	0.5 [0.5]	0.25	0.125	nt	4	0.25	0.5 [2]–8 (2)	0.13	8 (2)
17	4-HexylOPh-CH_2–	H	$NH(CH_2)_3NMe_2$	⩽0.125 [⩽0.125]	⩽0.125	⩽0.125	nt	16	⩽0.125 (2)	⩽0.125 [0.25]–16 (2)	⩽0.125	8–16 (2)
18	$CH_2CH(Bu)C_6H_{13}$	H	$NH(CH_2)_3NMe_2$	0.5 [1]	1	⩽0.125	nt	0.25	⩽0.125 (2)	0.25–8 (2) [nt]	⩽0.125	16 (1)
19	3-EtO-4*n*-C_6H_{13}O-Ph-CH_2–	H	$NH(CH_2)_3NMe_2$	⩽0.125 [0.25]	⩽0.125	⩽0.125	nt	2	⩽0.125 (2)	⩽0.125 [0.25]–16 (2)	⩽0.125	16 (2)
20	4-BuCH(Et)Oph-CH_2–	H	$NH(CH_2)_3NMe_2$	⩽0.125 [0.25]	⩽0.125	⩽0.125	nt	4	⩽0.125 (2)	⩽0.125–16 (2)	⩽0.125	16 (2)

( ), Number of tested microorganisms; [ ], MIC values for one strain in the presence of 30% bovine serum; nt, not tested.

Table 2. Dialkylated derivatives

ID				MIC or MIC range (mg/L)								
				S. aureus		*S. epidermidis*		*S. haemolyticus*	*E. faecalis*		*E. faecium*	
	R_1	R_2	R_3	Met-S (1)	Met-R (2)	Met-S (1)	Met-R (1)	(1)	Van-S (3)	Van-A (4)	Van-S (1)	Van-A (4)
21	*p*-Ph-$PhCH_2$–	*p*-Ph-$PhCH_2$–	OH	0.5 [64]	0.5	0.5	4	64	0.25–0.5	1–16 [>128]	0.5	4–>128
22	2-Naphthyl-CH_2–	2-Naphthyl-CH_2–	OH	2 [64]	1	2	16	16	⩽0.125	4–128 [>128]	2	2–>128
23	*p*-*n*Bu-Ph-CH_2–	*p*-*n*Bu-Ph-CH_2–	OH	2 [128]	2	4	8	8	0.25–1	2–4 [>128]	1	0.5–>128
24	*p*-*n*BuO-Ph-CH_2–	*p*-*n*BuO-Ph-CH_2–	OH	2 [32]	1	2	8	32	⩽0.125–0.5	1–8 [>128]	2	2–>128
25	*p*-PhOCO-Ph-CH_2–	*p*-PhOCO-Ph-CH_2–	OH	2 [64]	2	4	16	64	0.25–1	4 [>128]	1	2–>128
26	3-Indolyl-CH_2–	3-Indolyl-CH_2–	OH	4 [32]	2	64	128	>128	0.5–1	32–>128 [>128]	2	32–>128
27	*N*-*n*Bu-3-Indolyl-CH_2–	*N*-*n*Bu-3-Indolyl-CH_2–	OH	4 [4]	2	4	32	64	0.25	16–>128 [>128]	⩽0.125	1–>128
28	*N*-Bn-3-Indolyl-CH_2–	*N*-Bn-3-Indolyl-CH_2–	OH	⩽0.125 [4]	⩽0.125	⩽0.125	2	8	⩽0.125	2–>128 [>128]	⩽0.125	0.5–>128
29	*p*-Ph-$PhCH_2$–	*p*-Ph-$PhCH_2$–	$NH(CH_2)_3NMe_2$	1 [16]	1	0.25	1	2	0.25	1–16 [4]	0.5	2–32
30	2-Naphthyl-CH_2–	2-Naphthyl-CH_2–	$NH(CH_2)_3NMe_2$	1 [16]	2	⩽0.125	2	1	⩽0.125–0.25	0.5–64 [2]	0.25	0.25–32
31	*p*-*n*Bu-Ph-CH_2–	*p*-*n*Bu-Ph-CH_2–	$NH(CH_2)_3NMe_2$	1 [8]	2	0.25	2	2	⩽0.125–0.25	0.25–8 [8]	0.5	0.5–16
32	*p*-*n*BuO-Ph-CH_2–	*p*-*n*BuO-Ph-CH_2–	$NH(CH_2)_3NMe_2$	0.25 [2]	1	⩽0.125	0.5	1	⩽0.125	0.25–8 [1	0.125	0.125–16
33	*p*-PhOCO-Ph-CH_2–	*p*-PhOCO-Ph-CH_2–	$NH(CH_2)_3NMe_2$	1 [16]	1	0.25	2	2	⩽0.125–0.25	1–8 [4]	0.5	0.5–16
34	3-Indolyl-CH_2–	3-Indolyl-CH_2–	$NH(CH_2)_3NMe_2$	2 [4]	1	1	2	2	⩽0.125	0.5–8 [8]	0.25	0.25–32
35	*N*-*n*Bu-3-Indolyl-CH_2–	*N*-*n*Bu-3-Indolyl-CH_2–	$NH(CH_2)_3NMe_2$	0.5 [1]	2	0.25	2	0.25	⩽0.125–0.5	2–128 [2]	0.125	0.125–128
36	*N*-Bn-3-Indolyl-CH_2–	*N*-Bn-3-Indolyl-CH_2–	$NH(CH_2)_3NMe_2$	0.25 [1]	0.25	0.125	0.5	0.25	⩽0.125–0.25	4–32 [16]	0.125	0.125–32

( ), Number of tested microorganisms; [ ], MIC values for one strain in the presence of 30% bovine serum; nt, not tested.

the best results. The relevant role of hydrophobic chain the activity against resistant strains was confirmed by a lack of activity, against VRE, of the simple basic diamide of DA40 (Table 1, **14**).

N,N′-disubstituted derivatives of DA40 demonstrated high activity against susceptible enterococci and only modest activity against VRE (Table 2, **21–28**) but, as observed in the case of monoalkylated compounds, activity disappeared in the presence of serum. Again, the corresponding basic diamides increased the activity against VRE and revealed only moderate inactivation in the presence of serum (Table 2, **29–36**). In this class, the best compound appears to be **32**.

3. Conclusion

A40 and DA40 (**2**) are completely inactive against VRE. Mono- and dialkylation of the two amines slightly improved the antimicrobial activity. Basic diamides of N-mono- and N,N′-dialkylated derivatives did show emergence of any interesting activity against VRE. Among these diamides only the monoalkylated had good activity against the sensitive strain. In the presence of serum only, the diamides demonstrated activity against VRE. Compounds **18**, **19**, and **32** showed antimicrobial activity of particular interest and deserve further study.

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